

May 2025 Office of Chemical Safety and Pollution Prevention

Data Quality Evaluation Information for Environmental Hazard for Diethylhexyl Phthalate (DEHP) (1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 117-81-7



May 2025

This supplemental file contains information regarding the data quality evaluation results relevant to the analysis of environmental hazard for the *Draft Environmental Hazard Assessment for Diethylhexyl Phthalate (DEHP)*. EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '2021 Draft Systematic Review Protocol'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Draft Risk Evaluation for Diethylhexyl Phthalate (DEHP) – Systematic Review Protocol*.

Different data quality evaluation forms were used depending on the organism as described in the PECO statement in Appendix H.5.11 of the 2021 Draft Systematic Review Protocol. Each health outcome was evaluated independently within a given reference; therefore, each reference may have more than one overall quality determination (OQD) to more appropriately reflect the quality of each health outcome and the respective hazard endpoints as described by the study authors. Some data evaluation forms have general additional comments presented adjacent to the OQD to add further context. No OQD is determined for each reference as a whole, if it contains data from more than one evidence stream. The study details and respective endpoints are organized by first the relevant habitat (*i.e.*, aquatic, terrestrial), then taxa categories (*e.g.*, vertebrates, invertebrates, vegetation) followed by taxonomic groups (*e.g.*, fish, amphibian, mammalian, avian, worms, vascular plants), individual species, and finally exposure duration and health outcome (*e.g.*, mortality) categories relevant to the endpoint being evaluated.

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Reference

Diethylhexyl Phthalate

Habitat: Aquatic (freshwater)

Taxa: Vertebrates

	Carassius auratus		
1249842		Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.	21
2966358		Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology 163:16-26.	24
1333101		Pfuderer, P., Janzen, S., T, R.W., J. R. (1975). The identification of phthalic acid esters in the tissues of cyprinodont fish and their activity as heart rate depressors. Environmental Research 9(3):215-223.	30
	Clarias gariepinus		
4829324		Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.	32
	Clarius gariepinus		
5494023		Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharp- tooth catfish (Clarias gariepinus) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endrocrinology 254:22-37.	40
	Cyprinodon variegatus		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	45
	Cyprinus carpio		
5554274		Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. Journal of Environmental Sciences 46:47-54.	47
2510817		Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology 23(4):626-632.	50
2510817		Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology 23(4):626-632.	52
	Danio rerio		

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2298079		Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.	54
3350278		Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. Aquatic Toxicology 175:286-298.	56
2000753		Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (Danio rerio). Reproductive Biology 13(3):195-202.	60
5043619		Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Exam- ining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094.	64
5497528		Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.	71
	Gobiocypris rarus		
3071151		Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.	86
3071151		Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.	88
	Lampetra planeri		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	98
	Lepomis macrochirus		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	100
1316181		Bionomics, (1982). Bioassay report acute toxicity of compounds to bluegill (Lepomis macrochirus). Prepared by Bionomics Inc with cover letter.	102
1316201		Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus).	104
18064		Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bulletin of Environmental Contamination and Toxicology 26(4):446-452.	108
18050		Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (Lepomis macrochirus). :379-392.	110
	Leuciscus idus L.		
11328252		Kirsch, P., Munk, R. (1989). Report on the study of the acute toxicity.	112

Oncorhynchus mykiss

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5774391		Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.	114
11328250		Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a Flow-Through System.	116
11328250		Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a Flow-Through System.	119
5774391		Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.	125
	Oncorhynchus mykiss (Saln	no gairdneri)	
5530771		Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report) report no BW-83-3-1373.	129
	Oryzias latipes		
5774391		Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.	131
1303977		Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.	133
5489073		Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137.	135
1333890		Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, γ -hexachlorocyclohexane, and 17β -estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 18(1):9-12.	139
1337871		Shioda, T., Wakabayashi, M. (2000). Evaluation of reproductivity of medaka (Oryzias latipes) exposed to chemicals using a 2-week reproduction test. Water Science and Technology 42(7-8):53-60.	146
683795		Shioda, T., Wakabayashi, M. (2000). Effect of certain chemicals on the reproduction of medaka (Oryzias latipes). Chemosphere 40(3):239-243.	148
4728529		Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.	150
1334110		Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145.	158
5774391		Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.	164
1303977		Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.	168

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1333925		Metcalfe, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 20(2):297-308.	176
	Oryzias melastigma		
2298079		Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.	180
	Pelteobagrus fulvidraco		
4742097		Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84.	182
	Phoxinus phoxinus		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	194
	Pimephales promelas		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	196
1316188		Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.	200
1316189		Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions.	203
5774391		Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.	207
3071071		Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18.	209
3071071		Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18.	215
1014765		Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.	221
791717		Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.	227
	Poecilia reticulata		
697429		Zanotelli, V., Neuhauss, S., Ehrengruber, M. (2010). Long-term exposure to bis(2-ethylhexyl)phthalate (DEHP) inhibits growth of guppy fish (Poecilia reticulata). Journal of Applied Toxicology 30(1):29-33.	233

Pseudobagrus fulvidraco

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1335887		Jee, J. H., Koo, J. G., Keum, Y. H., Park, K. H., Choi, S. H., Kang, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetylcholinesterase activity in bagrid catfish, Pseudobagrus fulvidraco (Richardson). Journal of Applied Ichthyology 25(6):771-775.	235
	Pungitius pungitius		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	241
	Rana arvalis		
7328184		IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog, Rana arvalis.	243
7978546		IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.	251
5508563		Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmen- tal Toxicology and Chemistry 6(6):417-422.	283
	Rana chensinensis		
5493510		Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis tadpoles. Environmental Toxicology 33(1):112-121.	289
	Salmo gairdneri		
5353221		Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed zooplankton rich in wax esters. Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology 74(2):325-330.	295
791717		Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.	303
	Salmo mykiss		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	309
	Salmo Salar		
5646979		Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317.	311
5646979		Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317.	316
	Salmo salar		
5678430		Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.	324

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31448		Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxicology and Environmental Safety 8(1):55-74.	334
1	Taxa: Invertebrates		
	Aeshna sp.		
790132		Woin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination and Toxicology 38(2):220-225.	342
	Asellus breviicaudus		
1334646		Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	346
	Brachionus calyciflorus		
3070931		Cruciani, V., Iovine, C., Thomé, J. P., Joaquim-Justo, C. (2015). Impact of three phthalate esters on the sexual reproduction of the Monogonont rotifer, Brachionus calyciflorus. Ecotoxicology 25(1):192-200.	348
1336226		Zhao, L. L., Xi, Y. L., Huang, L., Zha, C. W. (2009). Effects of three phthalate esters on the life-table demography of freshwater rotifer Brachionus calyciflorus Pallas. Aquatic Ecology 43(2):395-402.	350
	Chironomus plumosus		
813673		Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40.	354
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	358
1334646		Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	362
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	364
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	368
813673		Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40.	370
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	374
	Chironomus riparius		
3859131		Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.	378
2519014		Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during Chirono- mus riparius development. Science of the Total Environment 470-471:1003-1011.	384

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681990	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in Chironomus riparius exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.	387
681634	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.	391
Chironomus tent	tans	
492760	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere 65(6):1074-1081.	397
1335360	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to Chironomus tentans.	401
674438	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environment International 33(6):817-822.	405
679311	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.	407
679312	Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., Vandeventer, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. Environmental Toxicology and Chemistry 20(8):1798-1804.	411
Daphnia magna		
1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	413
1316223	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to Daphnia magna (final report).	415
679904	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.	418
1334281	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426.	420
5750702	Huang, B., Li, D., Yang, Y. (2016). Joint toxicity of two phthalates with waterborne copper to Daphnia magna and Photobacterium phosphoreum. Bulletin of Environmental Contamination and Toxicology 97(3):380-386.	422
789536	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environ- mental Toxicology and Chemistry 22(12):3037-3043.	424
3070913	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna. Science of the Total Environment 545-546(Elsevier):127-136.	426
1335345	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna.	436
1335353	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna in the presence of fulvic acid.	438

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11328251	Muller (1983). Determination of the acute toxicity of di-2-ethylhexyl-phthlat (dehp) to the waterflea daphnia magna straus.	440
674438	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environment International 33(6):817-822.	442
2966135	Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49(12):7400-7410.	444
5043468	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total Environment 654:969-977.	449
5498837	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156.	459
1334646	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	471
679904	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.	473
1334281	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426.	479
1334646	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	485
1334951	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.	487
680120	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976.	495
5043468	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total Environment 654:969-977.	499
1316195	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95.	501
Dend	ocoelum lacteum	
59542	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	505
Gamm	aarus pseudolimnaeus	
1334646	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	507

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1334646		Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	509
	Gammarus pulex		
1335277		Oil,, Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp Gammarus pulex (L) to toxicants with cover letter.	511
732821		Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental Contamination and Toxicology 46(1):159-166.	513
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	519
	Helobdella sp.		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	521
	Hexagenia bilineata		
1334646		Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.	523
	Hyalella azteca		
679311		Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.	525
679312		Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., Vandeventer, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. Environmental Toxicology and Chemistry 20(8):1798-1804.	529
	Limnephilus sp		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	531
	Lumbriculus variegatus		
679312		Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., Vandeventer, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. Environmental Toxicology and Chemistry 20(8):1798-1804.	533
	Macrobrachium rosenbergii		
789598		Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.	535
	Paratanytarsus parthenogen	netica	
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	541

Table of Contents

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1335357		Monsanto, (1983). Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge Paratanytarsus parthenogenetica.	543
	Paratanytarsus parthenogeni	ca	
1316219		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424.	545
	Planorbis corneus		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	547
	Sialis sp.		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	549
	Tubijex sp.		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	551
Та	axa: Plants (Non-vascul	ar)	
	Chlorella vulgaris		
679344		Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in Chlorella vulgaris. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 42(2):179-183.	553
5692135		Shen, C., Wang, Y., Shen, Q., Wang, L., i, Lu, Y., Li, X., in, Wei, J., ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibi- tion and oxidative damage in the microalga Chlorella vulgaris. IOP Conference Series: Earth and Environmental Science 227(5):052054.	555
	Pseudokirchneriella subcapi	rata	
789536		Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environ- mental Toxicology and Chemistry 22(12):3037-3043.	559
	Selenastrium capricornutum		
1316196		Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga Selenastrum capricor- nutum.	561
	Selenastrum capricornutum		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	563
Т	axa: Plants (Vascular)		

Chara chara

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59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	565
	Lemna minor		
1340050		Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.	567
	Mentha aquatica		
59542		Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	569
	Spirodela polyrhiza		
1340050		Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.	571
	Triticum sp.		
3515118		Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428.	573
3515118		Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428.	575
Hał	oitat: Aquatic (mai	rine)	
]	Taxa: Vertebrates		
	Cyprinodon variegatus		
18110		Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.	581
	Oryzias melastigma		
2519010		Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquatic Toxicology 146:115-126.	583
	sheepshead minnow (Cypr	rinodon variegatus)	
1316224		Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report).	589
]	axa: Invertebrates		
	Haliotis diversicolor supe	rtexta	
697762		Liu, Y., Guan, Y., Yang, Z., Cai, Z., Mizuno, T., Tsuno, H., Zhu, W., Zhang, X. (2009). Toxicity of seven phthalate esters to embryonic development of the abalone Haliotis diversicolor supertexta. Ecotoxicology 18(3):293-303.	591

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Diethylhe	xyl Phthalate	Table of Contents	
1322103		Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta. Chinese Journal of Oceanology and Limnology 27(2):395-399.	593
1249532		Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environmental Pollution 159(5):1114-1122.	595
1322103		Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta. Chinese Journal of Oceanology and Limnology 27(2):395-399.	601
	Macrophthalmus japonicus		
5567571		Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, Macrophthalmus japonicus. Fish and Shellfish Immunology 87:322-332.	603
	Mysidopsis bahia		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	607
1316220		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).	609
	Mytilus edulis		
1334379		Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 2. The bioconcentration and depuration of di-2- ethylhexyl phthalate and diisodecyl phthalate in mussels, (Mytilus edulis). Chemosphere 11(4):427-435.	611
	PALAEMONETES PUGIO		
1333217		RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp Palaemonetes pugio (Holthuis). Water, Air, and Soil Pollution 9(3):323-336.	613
	Parvocalanus crassirostris		
3859142		Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethy- lene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.	619
3859142		Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethy- lene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.	623
3859142		Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethy- lene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.	629
	Penaeus vannamei		
679685		Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.	631

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Diethylho	exyl Phthalate	Table of Contents	
679685		Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.	633
Т	Faxa: Plants (Non-vascu	ılar)	
	Karenia brevis		
3230225		Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508.	639
Hab	oitat: Aquatic (brac	kish)	
1	Taxa: Vertebrates		
	Cyprinodon variegatus		
789995		Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.	642
	sheepshead minnow (Cypri	nodon variegatus)	
1316224		Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report).	644
Т	Faxa: Invertebrates		
	Artemia salina		
1315792		Sugawara, N. (1974). Toxic effect of a normal series of phthalate esters on the hatching of shrimp eggs. Toxicology and Applied Pharma- cology 30(1):87-89.	646
	Crassostrea virginica		
789995		Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.	648
	Eurytemora affinis		
679508		Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod Eurytemora affinis (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.	650
679508		Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod Eurytemora affinis (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.	654
	Mysidopsis bahia		
1316220		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).	656

Diethylh	exyl Phthalate	Table of Contents	
51937		Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.	658
	Penaecus aztecus		
789995		Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.	660
Hab	oitat: Terrestrial		
]	Faxa: Vertebrates		
	Bos taurus, Holstein Fresia	IN	
3071101		Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896.	662
	Common marmosets (Callia	thrix jacchus)	
630680		Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.	668
	Gallus domesticus		
683058		Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.	680
	Gallus gallus domesticus		
1249807		Abdul-Ghani, S., Yanai, J., Abdul-Ghani, R., Pinkas, A., Abdeen, Z. (2012). The teratogenicity and behavioral teratogenicity of di(2- ethylhexyl) phthalate (DEHP) and di-butyl Phthalate (DBP) in a chick model. Neurotoxicology and Teratology 34(1):56-62.	688
	Ovis aries		
2519005		Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.	696
	Putorius putorius		
746754		Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.	702
	Streptopelia risoria		
681729		Peakall, D. B. (1974). Effects of di-n-butyl and di-2-ethylhexyl phthalate on the eggs of ring doves. Bulletin of Environmental Contamina- tion and Toxicology 12(6):698-702.	710
	Sus domesticus		

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683666		Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.	712
	Sus scrofa		
683808		Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.	720
]	Faxa: Invertebrates		
	Caenorhabditis elegans		
5593882		How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.	726
5555457		Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266.	734
4728405		Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	743
698288		Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133.	751
5043459		Shin, N., Cuenca, L., Karthikraj, R., Kannan, K., Colaiácovo, M. P. (2019). Assessing effects of germline exposure to environmental toxicants by high-throughput screening in C. elegans. Pl o S Genetics 15(2):e1007975.	759
2215375		Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.	761
4829298		Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in Caenorhabditis elegans. Ecotoxicology and Environmental Safety 163:298-306.	779
5593882		How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.	785
4728405		Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	787
4728405		Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	789
	Drosophila melanogaster		
11784619		Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.	791
5495570		Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in Drosophila melanogaster. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.	796

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5495717	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila. Chemosphere 221:493-499.	802
5494836	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.	804
200657	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis 8(1):57-81.	813
5495717	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila. Chemosphere 221:493-499.	816
5494836	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.	820
11784619	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.	826
5494836	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.	828
11784619	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.	834
11784619	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.	848
Eisenia fetida		
3625226	Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm Eisenia fetida. Journal of Environmental Quality 14(3):383-388.	850
Folsomia fimetaria		
789786	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.	852
789786	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.	854
789786	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.	858
Lasius niger		
2345940	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110.	862
2347468	Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. Environmental Science and Pollution Research 21(23):13446-13451.	868

Diethylhe	exyl Phthalate	Table of Contents	
2345940		Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110.	870
	Spodoptera littoralis		
5494137		Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738.	872
Т	Faxa: Plants (Vascular)		
	Allium cepa		
1249401		Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the Allium cepa test. Mutation Research 743(1-2):20-24.	883
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	887
	Avena sativa		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	889
	Benincasa hispida		
2215486		Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant Benincasa hispida and its use for lowering DEHP content of intercropped vegetables. Journal of Agricultural and Food Chemistry 61(22):5220-5225.	895
	Cucumis sativus		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	899
1987637		Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di- (2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.	905
	Lolium perenne		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	909
	Medicago sativa		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	915
	Nicotiana tabacum		
5627041		Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination and seedling growth. Pedosphere 27(6):1073-1082.	921
	Nicotinana tobacum		

Diethylhe	xyl Phthalate	Table of Contents	
792357		Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-96.	925
	Raphanus sativus		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	933
	Triticum aestivum		
2915866		Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2- ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.	939
5493185		Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267.	945
	Triticum sp		
3350318		Gao, M., Qi, Y., Song, W., Xu, H. (2016). Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings. Chemosphere 151:76-83.	949
	Vigna radiata		
2510954		Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil. Pedosphere 24(1):107-115.	953

Study Citation:	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.					
Duration: Exposure Route, Media. Path:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; F Mechanistic- Di-ethylbexy	ish; <i>Carassius auratus</i> ; Not Applicable (e.g. Cell signaling/function-Endocrine toxicity- d phthalate (DEHP)	., fungi or algae Liver toxicolog	studies) or Not Reported y-Reproductive/Teratogenic-Nutritional and Metabolic		
HERO ID:	1249842	r phuhulute (DDTH)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Matria 1	Test Substance Identity	Low			
	Metric 1: Metric 2:	Test Substance Source	Low	The DEHP was identified by name only. The source of the DEHP was reported to be Sigma Aldrich, but it was not reported to be analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DEHP was not reported.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which the vehicle solvent, DMSO, was used at the same concentration as the exposure.		
	Metric 5:	Negative Control Response	High	Table 1 provides P-values for every treatment-to-treatment comparison for male livers and gonads; this includes the comparison between DEHP and the control. Figure 1 presents control response on the metabolic profiles of the liver and the gonads. Figure S4A provided heatmaps of control based z-scores of VIP>1 metabolites for both the liver and the gonad.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the goldfish were allocated to each tank. However, it was re- ported that each treatment group was randomly assigned to each tank to avoid bias.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	Study authors reported the test substances were added to the water every 24h after the tanks were drained to approximately 10% volume and then refilled with fresh water. Therefore the fish were exposed to declining concentrations of contaminants throughout each day of the study. Study authors also reported actual concentrations of the chemicals were not measured over time. A flow rate of 300mL/min of carbon-filtered City of Calgary water passed through each tank.		
	Metric 8:	Consistency of Exposure Administration	Low	The size of the test chambers was not reported, though holding/storage tanks were re- ported to be 49L. It was unclear if these were the same tanks used in the study. All fish were held at 17C. However, study authors admitted that the fish were exposed to declin- ing chemical concentrations over a 24h period until the solution was renewed. These test concentrations were not measured over time creating concern regarding the consistency of the study.		
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors reported that the test chemicals were not measured over time in the study and that the test chemicals would decline over a 24h period.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 10d. This appeared adequate to see a response.		
Continued on next page						

Environmental Hazard Evaluation

HERO ID: 1249842 Table: 1 of 1

Study Citation:	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chem	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	X 7 / 1 / T		c · 1			
Taxa, Species, Age:	Vertebrate; I	Fish; <i>Carassius auratus</i> ; Not Applicable (e.g	g., fungi or algae	studies) or Not Reported		
Health Outcome:	Mechanistic	-Cell signaling/function-Endocrine toxicity-	-Liver toxicology	-Reproductive/Teratogenic-Nutritional and Metabolic		
UEDO ID.	Di-ethylnexy	yi phthalate (DEHP)				
HERO ID:	1249842					
Domain		Metric	Rating	Comments		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level, as the goal of the study was to compare the response of different test chemicals to each other and a control as well as to a mixture of the test substances.		
	Metric 12:	Testing at or Below Solubility Limit	High	The DEHP exposure concentration was below the water solubility limit, and DMSO was used a vehicle solvent. An appropriate solvent control was used.		
Domain 4: Test Organi	sm					
	Metric 13:	Test Organism Characteristics	Medium	The goldfish were reported to be from Aquatic Imports in Calgary, Alberta, Canada. The age of the goldfish was not reported, though they were reported to be ~10cm long and 30g in weight.		
	Metric 14:	Acclimatization and Pretreatment	High	The fish were reported to be acclimated for 72h prior to the start of the study.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Study authors reported there were 20 fish per exposure aquaria. Only the male goldfish were used for this study, and there were 6-8 males in each tank. 6 males were used for assessment for each chemical. The treatment aquaria were not replicated due to practica limitations.		
Domain 5: Outcome A	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	The goldfish were fed daily for the duration of the holding and study period. They were held at 17C in 49L tanks for the acclimation period with 16-18 fish per tank. There were 20 fish in each exposure aquaria, but the size of the exposure aquaria was not reported. Carbon-filtered City of Calgary water was used in the study, but water quality parameters were not reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. H NMR was used to assess various changes in metabolite levels due to DEHP exposure in the male goldfish liver and gonads relative to the controls.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Detailed description of the metabolite extraction and H MNR spectroscopy were provided. Analysis methods of the data were also described in detail.		
Domain 6: Confoundin	a / Variable Co	ntrol				
Domain 0. Comoundin	Metric 19:	Confounding Variables in Test Design and Procedures	Low	More information on the actual study environmental conditions is needed, though the goldfish were reported to be acclimated to test conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal		

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Diethylhexyl Phthalate

O-PLS-DA analysis score plots were provided for treatment and control outcomes in Fig 1 as well as in Fig S3. Z-score plots on the effect of DEHP on each metabolite in comparison to the control was provided in Fig. S2. DEHP heatmaps in comparison to the control were provided in Fig. S4. Fig S5 B provides the SUS plots, and metabolite pathways affected by the treatments are provided in Fig S6. The effect on Canonical pathways is provided in Fig S7. Metabolic disturbances based on metabolites and their

		. continued from previ	ious page				
Study Citation:	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.						
Duration:	Overall Duration: 4 - 10 days; Exposure Duration	n: 4 - 10 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by	study authors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Carassius auratus; Not Applica	ble (e.g., fungi or algae	studies) or Not Reported				
Health Outcome:	Mechanistic-Cell signaling/function-Endocrine to	oxicity-Liver toxicology	y-Reproductive/Teratogenic-Nutritional and Metabolic				
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1249842						
Domain	Metric	Rating	Comments				
Domain 7: Data Presentation and Analysis							
	Metric 21: Statistical Methods	High	Detailed description of the data analysis was provided in 3 sections in the paper, "H NMR Data Analysis," "Statistical Analysis," and "Pathway Analysis."				

High

				superpathways is are provided in Fig 3. Canonical pathways and biological pathways that were affected by the exposure are provided in Fig. 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes.
Additional Comments:	This evaluati and quantific	on was on the effect of DEHP on the metabol ation. Mechanistic outcomes for metabolism	lic profiles of n , liver, reprodu	nale goldfish livers and testis. H NMR was used to assess metabolite identification ction, and endocrine were selected as the outcomes of interest.

Overall Quality Determination

Metric 22:

Reporting of Data

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Golshan, M.,	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M.						
	(2015). Di-(2	2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology						
Dentition	163:16-26.	163:16-26.						
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 2 .	I days					
Exposure Route,	Aquatic (fres	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	V (1 (F							
Taxa, Species, Age:	Vertebrate; F	ish; Carassius auratus; Adult						
Chamical	Developmen	//Growin						
UIEDO ID.	2066259	I philalate (DEHP)						
	2900338							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		_					
	Metric 1:	Test Substance Identity	Low	The chemical was only identified by name. CASRN, structure, or other chemical de- scriptors were not provided.				
	Metric 2:	Test Substance Source	Low	The test source was not reported, and the substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.				
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and the spacing of exposure levels were adequate.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms.				
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.				
	Metric 15:	Conditions Number of Organisms and	Low	The numbers of test organisms and replicates were lower than the typical number.				
		Replicates per Group						

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 2966358 Table: 1 of 3

		conth	nucu nom p			
Study Citation:	Golshan, M. (2015). Di-(Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology				
	163:16-26.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Carassius auratus; Adult					
Health Outcome:	Development/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2966358					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment methodology reported the intended outcome of interest.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures	e			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7. Data Present	ation and Anal	veie				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	The statistical methods were adequately described		
	Metric 22:	Reporting of Data	Medium	Brief results were described in the text. Data for the development/growth parameters are		
	Wieute 22.	Reporting of Data	Wiedrum	included in supplemental material that was not part of this pdf.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluati	ion is for the Development/Growth parame	ters (total ler	ngth, body mass, GSI, and HSI) assessed.		
Overall Quali	ty Detern	nination	High			

continued from previous page

Diethylhexyl Phthalate

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HERO ID: 2966358 Table: 2 of 3

Study Citation:	Golshan, M.	, Hatef, A., Socha, M., Milla, S., Butts, I.	A., Carnevali	, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M.
Duration: Exposure Route, Media. Path:	(2015). Di-(163:16-26. Overall Dura Aquatic (free	2-ethylhexyl)-phthalate disrupts pituitary ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	and testicular 1 days authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; F Reproductive Di-ethylhexy	Fish; <i>Carassius auratus</i> ; Adult e/Teratogenic yl phthalate (DEHP)		
Domain	2900338	Metric	Rating	Comments
Domain 1: Test Substand	ce	moure	Runng	conments
	Metric 1:	Test Substance Identity	Low	The chemical was only identified by name. CASRN, structure, or other chemical de- scriptors were not provided.
	Metric 2:	Test Substance Source	Low	The test source was not reported, and the substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and the spacing of exposure levels were adequate.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organisi	n			
-	Metric 13:	Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms.
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
		Cont	inued on nex	t page

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Environmental Hazard Evaluation

HERO ID: 2966358 Table: 2 of 3

		contir	ued from p	previous page			
Study Citation:	Golshan, M. (2015). Di-(163:16-26	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology 163:16-26					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	A						
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Carassius auratus; Adult					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	2966358						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment methodology reported the intended outcome of interest.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	The statistical methods were adequately described.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quali	ty Detern	nination	High				

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HERO ID: 2966358 Table: 3 of 3

Study Citation:	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology					
Duration:	163:16-26. Overall Dur	ation: > 21 days. Exposure Duration: > 2	1 days			
Exposure Route.	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media. Path:						
Taxa, Species, Age:	Vertebrate; Fish; Carassius auratus; Adult					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2966358					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.		
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
		Administration	C			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and the spacing of exposure levels were adequate.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4: Test Organic	m					
Domain 4. 10st Organis	Metric 13.	Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms		
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions		
	Wieure 14.	Conditions	Ingn	The test organisms were acclimatized to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.		
Domain 5: Outcome As	sessment		TT' 1			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
		Cont	tinued on nex	at page		

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Diethylhexyl Phthalate

	continued from previous page						
Study Citation:	Golshan, M.	, Hatef, A., Socha, M., Milla, S., Butts, I. A	., Carnevali	, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M.			
	(2015). Di-((2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology					
Derections	163:16-26.	163:16-26.					
Duration:	A quotio (from	(1001: > 21) days; Exposure Duration: > 21	days	abamical of interact in averaging water but weakle to determine averation take route)			
Exposure Koule,	Aquatic (free	sinvater); water; not determined by study a	utions (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake foute)			
Toyo Species Ages	Vantahnata, I	Tich Canagaing annature Adult					
Taxa, Species, Age:	Vertebrate; f	Alsn; Carassius auratus; Adult Biomoritors (opposite and affect) Call size	alin a/fun at	an Constant (including DNA remain)			
Chamical	Di athulhaur	-biomarkers (exposure and effect)-Cell sign	lanng/runcu	on-Genolox (including DNA repair)			
	2066358	(DEHF)					
	2900338						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	The outcome assessment methodology reported the intended outcome of interest.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	The statistical methods were adequately described.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
		* *		<u>۸</u>			
Additional Comments:	None						
Overall Ouali	tv Deterr	nination	High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Pfuderer, P.,	Pfuderer, P., Janzen, S., T, R.W., , J. R. (1975). The identification of phthalic acid esters in the tissues of cyprinodont fish and their activity as heart rate						
Duration:	depressors. 1 Overall Dura	depressors. Environmental Research 9(3):215-223. Overall Duration: Not-reported; Exposure Duration: Not-reported						
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	uthors (i.e., chemical of int	erest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa Species Age:	Vartabrata: I	Vertebrate: Fish: <i>Carassius auratus</i> : Invenile						
Health Outcome:	Cardiovascu	Cardiovascular						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	1333101							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.				
Domain 2: Test Design								
U	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3. Exposure Ch	oractorization							
Domain 3. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.				
	Metric 8:	Consistency of Exposure Administration	Uninformative	It was reported that a sonicated emulsion was used for exposure, and exposure admin- istration cannot be administered consistently across test organisms/study groups using emulsions.				
	Metric 9:	Measurement of Test Substance Concentration	Uninformative	Exposure concentrations were not measured, and nominal values are highly uncertain due to the nature of the test substance.				
	Metric 10:	Exposure Duration and Frequency	Uninformative	The duration of exposure and/or exposure frequency were not reported.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and the spacing of ex- posure levels. It was reported "no concentration of DEHP tried was effective," and the concentrations specified on Fig 6 are not legible.				
	Metric 12:	Testing at or Below Solubility Limit	Low	An emulsion was used so there is a high probability it exceeds solubility.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates were not reported. The figure on heart rate activity (Fig 6) showed only the response of an individual fish. The cited reference for methods (Francis et al., 1975) was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.				
		(Continued on next page					

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Diethylhexyl Phthalate

HERO ID: 1333101 Table: 1 of 1

		coi	ntinued from previous	page			
Study Citation:	Pfuderer, P., depressors. I	Pfuderer, P., Janzen, S., T, R.W., J. R. (1975). The identification of phthalic acid esters in the tissues of cyprinodont fish and their activity as heart rate depressors. Environmental Research 9(3):215-223.					
Duration:	A quetia (free	Aguatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Exposure Route, Madia Dath.	Aquatic (free	Aquatic (neshwater); water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa Snecies Age	Vertebrate: F	Vertebrate; Fish; Carassius auratus; Juvenile					
Health Outcome:	Cardiovascu	Cardiovascular					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1333101	()					
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown, but the results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) or sufficient information to determine if excessive variability or unexpected outcomes occurred.			
Additional Comments:	Overall, a poorly designed and poorly written study. Negative control group was not reported. Details of the experimental system, test media preparation, exposure concentrations, duration of exposure were not provided. It was reported that a sonicated emulsion was used for exposure. Mean heart rate data was not provided for DEHP, and the figure that shows the heart rate activity of one individual fish was of poor resolution. The cited reference for methods was not available at the time of review.						
Overall Qualit	ty Detern	nination	Uninformative	e			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Adeogun, A African shar Pharmacolos	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days				
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study	authors (i.e., chemical of int	erest in exposure water, but unable to determine exact uptake route)			
Media. Path:		require (resirvater), water, rot determined by study autions (r.e., enemiear of incress in exposure water, but anable to determine exact aptake route)					
Tava Species Age	Vertebrate: F	Vertebrate: Fish: Clarias agrianinus: Iuvenile					
Health Outcome	ADME (biot	transformation)					
Chamical	Di athulhavi	al phthalata (DEHD)					
	4820224	(DEHF)					
	4829324						
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	nce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
	Wietrie 0.	Randoniized Anocation	Wiedrum	The study reported that organisms were randomly anocated into study groups.			
Domain 3: Exposure C	haracterization						
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured other than in tissue.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
			C				
Domain 4: Test Organis	sm						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were the same for the control and the exposed organisms,			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.			
			Continued on next page	•			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18. **Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Clarias gariepinus; Juvenile Health Outcome: ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 4829324 Metric Rating Comments Domain Metric 17: Outcome Assessment Methodology Low The outcome assessment methodology was not clearly reported. Metric 18: Consistency of Outcome Low Details regarding the execution of the study protocol for outcome assessment were limited. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures Outcomes Unrelated to Exposure High Metric 20: There were no differences among groups. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Uninformative Statistical analysis was not conducted. Metric 22: Reporting of Data Low Data for exposure-related findings were not reported for each treatment and control group. Explanation of Unexpected Outcomes Metric 23: High There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination

Uninformative

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HERO ID: 4829324 Table: 2 of 4

Study Citation:	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and						
Duration:	Pharmacolog Overall Dura	Pharmacology 213:7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route, Media Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate: F	Vertebrate: Fish: <i>Clarias gariepinus</i> : Juvenile					
Health Outcome:	Development/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	4829324						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	Metric 8.	Consistency of Exposure	Medium	Reporting omissions were unlikely to have a substantial impact on results			
	inetite o.	Administration	meanum	reporting offissions were unitery to have a substantial impact of results.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a			
		Spacing of Exposure Levels	0	dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m Motria 12:	Tost Organism Characteristics	High	The test appendixes years adaptative described and years abteined from a well-bla accurate			
	Metric 14	Acclimatization and Pretreatment	High Medium	All pretreatment conditions were the same for the control and the averaged organisms			
	wieute 14.	Conditions	wiedium	although few details were provided.			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects.			
Damain 5: 0 (
Domain 5: Outcome Ass	Sessment Matria 16:	A deguage of Test Conditions	Madium	Environmental conditions of the test system were accentable for the maintenents of			
	wieuric 10:	Adequacy of Test Conditions	Medium	construction of the test system were acceptable for the maintenance of organism health, although few details were provided			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
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Diethylhexyl Phthalate

		contir	iuea from p	previous page		
Study Citation:	Adeogun, A African shar Pharmacolog	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Clarias gariepinus; Juvenile				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	4829324					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	High			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 4829324 Table: 3 of 4

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair) Di-ethylhexyl phthalate (DEHP)					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice		6			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.		
Domain 2: T+ D						
Domain 2: Test Design	Matria 4.	Nagativa Controla	High			
	Metric 4:	Negative Control Response	High	The biological response of the pagetive concurrent negative control group.		
	Metric 5.	Pandomized Allocation	Medium	The biological response of the negative control group was suitable.		
	Wieute 0.	Kandoniized Anocation	Wiedrum	The study reported that organisms were failed into study groups.		
Domain 3. Exposure Ch	naracterization					
Domain 5. Exposure of	Metric 7:	Experimental System/Test Media	Medium	Reporting omissions were unlikely to have a substantial impact on results.		
		Preparation	1,100,000,000			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions were unlikely to have a substantial impact on results.		
		Administration				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a		
	Weute 11.	Spacing of Exposure Levels	mgn	dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
	· · · ·		0	L d		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were the same for control and exposed organisms, although		
	Matria 15.	Conditions Number of Organisms and	Modium	Iew details were provided.		
	Metric 15:	Replicates per Group	wiedium	ine numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Oroup				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	The environmental conditions of the test system were acceptable for the maintenance of		
				organism health, although few details were provided.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Continued on next page						
PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Diethylhexyl Phthalate

		contir	nued from p	previous page		
Study Citation:	Adeogun, A African shar Pharmacolog	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Clarias gariepinus; Juvenile					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	4829324					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Deterr	nination	High			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 4829324 Table: 4 of 4

Study Citation:	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.						
Duration: Exposure Route, Media Path	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa. Species. Age:	Vertebrate: I	Vertebrate; Fish; Clarias gariepinus; Juvenile					
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Di-ethylhex	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	4829324						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	M-4	Preparation	Madian				
	Metric 8:	Administration	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a			
		Spacing of Exposure Levels		dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
5	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were the same for the control and the exposed organisms, although few details were provided.			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects.			
Domain 5: Outcome As	sessment	A dequeer of Test Car ditions	M 1				
	Metric 16:	Adequacy of fest Conditions	Medium	The environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
		Cont	tinued on nex	xt page			

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Diethylhexyl Phthalate

		conur	iuea irom p	brevious page			
Study Citation:	Adeogun, A African shar Pharmacolog	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish (Clarias gariepinus) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Clarias gariepinus; Juvenile					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	4829324						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Oualit	tv Deterr	nination	High				

atinued fro m nrovio

Study Citation:	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish					
Duration: Exposure Route, Modia Path:	(Clarias gariepinus) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endrocrinology 254:22-37. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate: F	ish: Clarius gariepinus: Iuvenile				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5494023					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.		
	Metric 2:	Test Substance Source	High	The source of the DEHP was reported to be from Tokyo Chemical Industry in Japan, and it was verified by GC-MSD.		
	Metric 3:	Test Substance Purity	High	The DEHP purity was reported to be 99%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used. Ethanol was the reported vehicle solvent in the treatments.		
	Metric 5:	Negative Control Response	High	The negative control response was reported and adequate for the outcomes of interest. The negative control responses can be found in the text in section 3.1.		
	Metric 6:	Randomized Allocation	Medium	It was reported the fish were randomly allocated into 10 120L tanks with 60L of dechlo- rinated tap water with 50 fish per exposure group.		
Demain 2: Error ch						
Domain 3: Exposure Ch	Matria 7:	Experimental System/Test Media	Uich	The system was reported to be a static reported system with the test medium reported		
	Mette 7.	Preparation	Ingn	every third day for the duration of the study. The stock solution was prepared by dissolv- ing 10mL of DEHP in 90mL of ethanol to get 109.5g/L DEHP. Test concentrations were prepared through serial dilutions with dechlorinated tap water.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were in 120L tanks with 50 fish per tank. Each tank had 60L of test solu- tion that was diluted with dechlorinated tap water. All tests were conducted at 24C.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentrations were measured at any point in the study.		
	Metric 10:	Exposure Duration and Frequency	High	The study duration was reported to be 14 days. This was adequate to observe a response.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 4 exposure groups. The spacing was adequate to see a response in DEHP accumulation in the liver according to dose.		
	Metric 12:	Testing at or Below Solubility Limit	High	The highest concentration tested was above the water solubility limit of DEHP. How- ever, ethanol was used as a vehicle solvent, and the solvent control results were ade- quate.		
Domain 1. Test Organis	n					
Domanii 4. Test Olganisi	Metric 13:	Test Organism Characteristics	High	The C. gariepinus were reported to be from the hatchery unit of the Aquaculture Depart- ment at the University of Ibadan. They were reported to be fingerlings that were 4 weeks old.		
		Cont	inued on nex	t page		

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Environmental Hazard Evaluation

HERO ID: 5494023 Table: 1 of 2

Study Citation: Aruk (Clar Duration: Over Exposure Route, Aqua Media, Path: Taxa, Species, Age: Verte Health Outcome: ADM Chemical: Di-et HERO ID: 5494 Domain Metr Domain 5: Outcome Assessment Metr Metr Metr Metr	twe, A., rias garie all Durat atic (fresl ebrate; Fi AE (biotr thylhexy: 023 ic 14: ic 15: nt ic 16:	Ibor, O. R., Adeogun, A. O. (2017). Bip epinus) exposed to waterborne di-(2-ethylh tion: 11 - 21 days; Exposure Duration: 11 hwater); Water; Not determined by study a ish; <i>Clarius gariepinus</i> ; Juvenile ransformation) 1 phthalate (DEHP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	phasic modu lexyl) phthala - 21 days authors (i.e., Rating High Low	Ilation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfis ate. General and Comparative Endrocrinology 254:22-37. chemical of interest in exposure water, but unable to determine exact uptake route) Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Duration: Over Exposure Route, Aqua Media, Path: Taxa, Species, Age: Verte Health Outcome: ADM Chemical: Di-et HERO ID: 5494 Domain Metr Domain 5: Outcome Assessme Metr	rall Durat atic (frest bebrate; Fi AE (biotr thylhexy: 023 ic 14: ic 14: ic 15: nt ic 16:	tion: 11 - 21 days; Exposure Duration: 11 hwater); Water; Not determined by study a ish; <i>Clarius gariepinus</i> ; Juvenile ransformation) l phthalate (DEHP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	- 21 days authors (i.e., Rating High Low	chemical of interest in exposure water, but unable to determine exact uptake route) Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Exposure Route, Aqua Media, Path: Taxa, Species, Age: Verte Health Outcome: ADM Chemical: Di-et HERO ID: 5494 Domain Metr Domain 5: Outcome Assessmen Metr	atic (frest ebrate; Fi AE (biotr thylhexy: 023 ic 14: ic 15: nt ic 16:	hwater); Water; Not determined by study a ish; <i>Clarius gariepinus</i> ; Juvenile ransformation) l phthalate (DEHP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	chemical of interest in exposure water, but unable to determine exact uptake route) Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Media, Path: Faxa, Species, Age: Verte Health Outcome: ADN Chemical: Di-et HERO ID: 5494 Domain Metr Metr Metr Domain 5: Outcome Assessment Metr Metr Domain 5: Metr	ebrate; Fi AE (biotr thylhexy: 023 ic 14: ic 15: nt ic 16:	ish; <i>Clarius gariepinus</i> ; Juvenile ransformation) l phthalate (DEHP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Taxa, Species, Age: Verte Health Outcome: ADM Chemical: Di-et HERO ID: 5494 Domain Metr Metr Metr Domain 5: Outcome Assessmen Metr Metr Metr Metr Metr Domain 5: Outcome Assessmen Metr	AE (biotrithylhexy) 023 ic 14: ic 15: nt ic 16:	ish; <i>Clarius gariepinus</i> ; Juvenile ransformation) l phthalate (DEHP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Health Outcome: ADM Chemical: Di-et HERO ID: 5494 Domain Metr Metr Domain 5: Outcome Assessmen Metr	AE (biotr thylhexy 023 ic 14: ic 15: nt ic 16:	Ansformation) I phthalate (DEHP) Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Chemical: Di-et HERO ID: 5494 Domain Metr Domain 5: Outcome Assessmer Metr Metr	thylhexy 023 ic 14: ic 15: nt ic 16:	l phthalate (DEHP) Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
HERO ID: 5494 Domain Metr Domain 5: Outcome Assessmen Metr Metr	023 ic 14: ic 15: nt ic 16:	Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Domain Metr Metr Domain 5: Outcome Assessmer Metr Metr	ic 14: ic 15: nt ic 16:	Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating High Low	Comments The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Metr Metr Domain 5: Outcome Assessme Metr Metr	ic 14: ic 15: nt ic 16:	Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	High Low	The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study. It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Metr Domain 5: Outcome Assessmer Metr Metr	nt ic 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Domain 5: Outcome Assessme Metr	nt ic 16:	Repleates per Gloup		·1 ····· · · · · · · · · · · · · · · ·
Domain 5: Outcome Assessmer Metr	nt ic 16:			
Metr. Metr	ric 16:			
Metr		Adequacy of Test Conditions	Medium	The fish were held at 24C with a pH of 7 and a DO of 3mg/L. A natural photoperiod was reported to be used, but was not specified. The fish were fed twice daily throughout the experiment. Other properties of the test water were not reported.
	ic 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DEHP accumulation in the liver of treated fish.
Metr	ic 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. EPA DIN 19742 was cited for the methodology for determining tissue levels. This method used was gas chromatography/mass selective detector for assessment.
Domain 6: Confounding / Varia	able Con	trol		
Metr	ric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment
Metr	ric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7. Data Presentation a	nd Anals	zeie		
Metr.	ic 21:	Statistical Methods	High	All data were presented as +- mean standard deviation and were analyzed using Prism GraphPad 5. Significant differences between the solvent control and exposure groups were performed using one-way ANOVA. Statistical differences were analyzed using Tukey's Multiple Comparison Test
Metr	ric 22:	Reporting of Data	Low	Results were presented in the text for the exposure and the control responses in section 3.1.
Metr	ric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported for the DEHP accumulation in the liver.
Additional Comments: This	portion of	of the evaluation was on the accumulation	of DEHP in	the liver after 14 days of exposure. ADME was selected as the outcome of interest.

Study Citation:	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish (Clarias gariepinus) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endrocrinology 254:22-37.						
Duration: Exposure Route, Media Path	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile Mechanistic-Cell signaling/function-Endocrine toxicity-Neurotoxicology Di-ethylhexyl phthalate (DEHP) 5494023						
Domain	Metric Rating Comments						
Domain 1: Test Substan	ce						
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High High	The DEHP was identified by CASRN. The source of the DEHP was reported to be from Tokyo Chemical Industry in Japan, and it was verified by GC-MSD.			
	Metric 3:	Test Substance Purity	High	The DEHP purity was reported to be 99%.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used. Ethanol was the reported vehicle solvent in the treatments.			
	Metric 5:	Negative Control Response	High	The negative control response was reported and adequate for the outcomes of interest. The negative control responses can be found in Figures 2-10.			
	Metric 6:	Randomized Allocation	Medium	It was reported the fish were randomly allocated into 10 120L tanks with 60L of dechlo- rinated tap water with 50 fish per exposure group.			
Domain 3: Exposure Ch	aracterization						
Domain J. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The system was reported to be a static renewal system with the test medium renewed every third day for the duration of the study. The stock solution was prepared by dissolving 10mL of DEHP in 90mL of ethanol to get 109.5g/L DEHP. Test concentrations were prepared through serial dilutions with dechlorinated tap water.			
	Metric 8:	Consistency of Exposure	High	All exposures were in 120L tanks with 50 fish per tank. Each tank had 60L of test solution that was diluted with dechlorinated tap water. All tests were conducted at 24C.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentrations were measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The study duration was reported to be 14 days. This was adequate to observe a response.			
	Metric 11:	Number of Exposure Groups/	High	There were 4 exposure groups. The spacing was adequate to see a biphasic response.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The highest concentration tested was above the water solubility limit of DEHP. How- ever, ethanol was used as a vehicle solvent, and the solvent control results were ade- quate.			
Domain 4: Test Organis:	m Metric 13:	Test Organism Characteristics	High	The C. gariepinus were reported to be from the hatchery unit of the Aquaculture Depart- ment at the University of Ibadan. They were reported to be fingerlings that were 4 weeks			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study.			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 5494023 Table: 2 of 2

		cont	tinued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish (Clarias gariepinus) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endrocrinology 254:22-37. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Clarius gariepinus; Juvenile					
Health Outcome:	Mechanistic-Cell signaling/function-Endocrine toxicity-Neurotoxicology						
Chemical: HERO ID:	Di-ethylhexyl phthalate (DEHP) 5494023						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The fish were held at 24C with a pH of 7 and a DO of 3mg/L. A natural photoperiod was reported to be used, but was not specified. The fish were fed twice daily throughout the experiment. Other properties of the test water were not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-the gene expression of acute steroidogenic pathways in the brain and kidneys, as well as hormone levels.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fish were sampled on days 3, 7, and 14, and head kidney and brain samples were harvested and snap frozen in liquid nitrogen and preserved at -80C until processing. Real-time PCR was used for gene expression analysis. Steroid hormone analysis was performed using EIA kits. Immunochemical analysis was performed according to Bradford (1976).			
Domain 6: Confoundir	og / Variable Co	ntrol					
Domain 0. Comoundin	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Prasar	ntation and Anal	veic					
Domain 7. Data Pleser	Metric 21:	Statistical Methods	High	All data were presented as +- mean standard deviation and were analyzed using Prism			

Additional Comments: This portion of the evaluation was on the effect of DEHP on gene expression of the steroidogenic pathways of the kidney head and the brain. Several genes in these pathways were measured and a biphasic response was observed. Samples were taken on days 3, 7, and 14. Hormone levels were also assessed. Mechanistic outcomes for neurotoxicity, endocrine toxicity, and cell signaling and function were chosen as the outcomes of interest.

High

High

GraphPad 5. Significant differences between the solvent control and exposure groups were performed using one-way ANOVA. Statistical differences were analyzed using

Data for the exposure and the control responses were reported in Figures 2-10 and dis-

Study authors did not report any unexpected outcomes. Variation was reported in the

played the biphasic response. Table 2 displayed hormone concentrations.

Tukey's Multiple Comparison Test.

figures and the tables.

Continued on next page ...

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Metric 22:

Metric 23:

Reporting of Data

Explanation of Unexpected Outcomes

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5494023 Table: 2 of 2

		continued from previous page	
Study Citation:	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro	- and interrenal steroidogenesis in juvenile African sharptooth catfish
	(Clarias gariepinus) exposed to waterborne d	li-(2-ethylhexyl) phthalate. General and	Comparative Endrocrinology 254:22-37.
Duration:	Overall Duration: 11 - 21 days; Exposure Du	aration: 11 - 21 days	
Exposure Route,	Aquatic (freshwater); Water; Not determined	by study authors (i.e., chemical of inte	erest in exposure water, but unable to determine exact uptake route)
Media, Path:			
Taxa, Species, Age:	Vertebrate; Fish; Clarius gariepinus; Juvenil	e	
Health Outcome:	Mechanistic-Cell signaling/function-Endocri	ne toxicity-Neurotoxicology	
Chemical:	Di-ethylhexyl phthalate (DEHP)		
HERO ID:	5494023		
Domain	Metric	Rating	Comments

Domain	Metric	Rating	Comments	
Overall Quality De	termination	High		

Study Citation:	Adams, W. J	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic						
Duration	organisms. E	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574. Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	1	······ , ····· ; ····· ; ····· ;		· · · · · · · · · · · · · · · · · · ·				
Taxa, Species, Age:	Vertebrate; F	Fish; Cyprinodon variegatus; Juvenile						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	1321996							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		Ŧ					
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.				
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number not provided. No analytical data was reported.				
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.				
Domain 2: Test Design	N . · · ·		TT' 1					
	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5:	Regarized Allocation	High	The control response was acceptable.				
	Metric 0:	Randomized Anocation	LOW	The allocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test				
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.				
	Metric 11:	Number of Exposure Groups/	High	The exposure levels were appropriate. A range finding test was performed.				
		Spacing of Exposure Levels	U					
	Metric 12:	Testing at or Below Solubility Limit	High	The test performed was at or below the water solubility limit.				
Domain 4: Test Organia	m							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source was not reported				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.				
		Conditions	111511	in appropriate accontation period for the test was reported.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.				
Domain 5: Outcome Ass	sessment							

Continued on next page ...

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Diethylhexyl Phthalate

		conti	nued from p	previous page			
Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic			
Dennetterne	organisms. E	Environmental Toxicology and Chemistry I	4(9):1569-1	5/4.			
Duration:	Overall Duration: 0 - 4 days (0-9011), Exposure Duration: 0 - 4 days (0-9011) A quetie (freshwater): Water: Net determined by study outbors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Exposure Koule,	Aquatic (Ireshwater); water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	N 7 (1 (F						
Taxa, Species, Age:	Vertebrate; Fish; Cyprinodon variegatus; Juvenile						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.			
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.			
Domain 6: Confounding	Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.			
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Quality Determination		High					

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. Journal of Environmental Sciences 46:47-54. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Cyprinus carpio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 5554274					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	Just the chemical name was provided.		
	Metric 2:	Test Substance Source	Low	There was no analytical verification information provided for the test DEHP. The source was listed as TCI, Tokyo.		
	Metric 3:	Test Substance Purity	Low	The purity of the DEHP used in the test was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A control group was used in this test.		
	Metric 5:	Negative Control Response	Medium	Control responses (threshold cycles) were shown in the expression variability of ref- erence genes Fig. 2. However, it was not mentioned in the study whether those values shown were representative of an adequate control response.		
	Metric 6:	Randomized Allocation	Low	Authors did not report how oocytes were allocated into test exposures.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	There is very minimal information on how the test substance was prepared and the oocyte exposure. DEHP is not very water soluble, but there was no mention of whether a solvent was used to get it into test solution. There were a couple studies referenced in the methods section of this paper, and one of those papers said they used EtOH as a solvent to get DEHP into the test solutions. The paper did not report any measured test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Medium	The paper does not indicate if there is a difference in exposure administration between the control and treatment groups. For the oocyte chemical exposure, there was no infor- mation given on environmental conditions or details on the exposure vessels used, so it is unclear whether there could have been inconsistencies in these aspects of the study. The molecular analyses methods seemed to be administered consistently for all samples.		
	Metric 9:	Measurement of Test Substance Concentration	Low	The test concentrations of DEHP used in the incubation of oocytes were not specifically reported. It was mentioned in the methods section that 0.1, 1, and 5 u mol/L DEHP were the concentrations used for the exposure groups.		
	Metric 10:	Exposure Duration and Frequency	High	The incubation duration seemed appropriate based on the results obtained. In the meth- ods section this paper referenced a couple other papers doing similar studies with DEHP. Specific results for relative expression level per timepoint (hours after incubation) were not shown for DEHP.		
		Con	ntinued on next pa	ge		

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Environmental Hazard Evaluation

HERO ID: 5554274 Table: 1 of 1

Study Citation:	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by					
Dermetterne	diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. Journal of Environmental Sciences 46:47-54.					
Duration:	Overall Duration: 0 - 4 days (0-90n); Exposure Duration: 0 - 4 days (0-90n) Aquatic (freshwater): Cell Culture Media: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Exposure Koule, Modio Both	Aquate (freshwater); Cen Cutture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate: Fish: <i>Cynrinus carnio</i> : Not Applicable (e.g., fungi or algae studies) or Not Reported					
Tava Spacias Agas						
Iaxa, Species, Age:	Machanistia	Parroductive/Terrotogonia	Tungi or argae si	iudies) of Not Reported		
Chamicali	Di athulhavi	-Reproductive/ relatogenic				
HERO ID.	5554274	yi pitilalate (DEIII)				
	5554274					
Domain	Matria 11:	Metric	Rating	Comments		
	Metric 11:	Spacing of Exposure Levels	IN/A	The methods section indicated infee concentrations of DEHP used in the exposure.		
		Spacing of Exposure Levels		bating oocytes to different concentrations seemed irrelevant to the molecular analyses of interest.		
	Metric 12:	Testing at or Below Solubility Limit	Low	It is unclear from the text in the methods section whether a solvent was used to get DEHP into solution for the different concentration exposures.		
Domain 4: Test Organis	sm					
	Metric 13:	Test Organism Characteristics	Medium	The study reported that gravid adult female common carp were obtained from a local		
				aquatic market in Xiamen. No information was given on what water source they were harvested in. These fish oocytes were removed for use in the exposure study.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study authors did not mention specific pre-treatment conditions for the oocytes other than saying once they were removed from the fish, they were then incubated in Cort-		
	34.1.15			land's solution, and the pH was adjusted to 7.4.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each experimental sample was amplified in triplicate. The Ct values (per reference gene) determined by qPCR were based off of an n=15.		
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	Not much was mentioned on the environmental conditions during the incubation period of oocytes in DEHP concentrations. Oocytes were incubated in Cortland's solution containing streptomycin and penicillin and various nutrients. The cDNA synthesized after the RNA extraction was stored at -20C until use in the qPCR. The qPCR was run under appropriate conditions to get absolute expression value (threshold cycles) results.		
	Metric 17:	Outcome Assessment Methodology	High	The methods to extract RNA from the exposed oocytes and then synthesize cDNA from that were adequately described. The primer design was sufficient and the qPCR analyses were adequate to obtain the expression profiles of the reference genes.		
	Metric 18:	Consistency of Outcome Assessment	High	There was no information in the study to indicate any differences in molecular analyses among control and treatment groups.		
Domain 6: Confoundin	σ / Variable Co	ntrol				
2 children of Comoundury	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There was not enough information on environmental conditions throughout the incu- bation period for the oocytes to say whether there were any confounding variables or differences between treatment groups		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among treatment groups.		
Domain 7: Data Presen	tation and Anal	ysis				

Diethylhexyl Phthalate

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5554274 Table: 1 of 1

	,	continued from previous page				
Study Citation:	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). R	eference gene validation for quantification	ation of gene expression during final oocyte maturation induced by			
-	diethylstilbestrol and di-(2-ethylhexyl)-phthala	te in common carp. Journal of Environ	mental Sciences 46:47-54.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure	Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Cell Culture Media; Not	t determined by study authors (i.e., ch	emical of interest in exposure water, but unable to determine exact			
Media, Path:	uptake route)					
Taxa, Species, Age:	Vertebrate; Fish; Cyprinus carpio; Not Applica	ble (e.g., fungi or algae studies) or Not	Reported			
Health Outcome:	Mechanistic-Reproductive/Teratogenic	Mechanistic-Reproductive/Teratogenic				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5554274					
Domain	Metric	Rating	Comments			

Domain		Metric	Rating	Comments
	Metric 21:	Statistical Methods	High	The study reported using SPSS 20.0 software for statistical analysis. One-way ANOVA was used to compare relative expression levels and Tukey's post-hoc was used for comparisons against zero hour.
	Metric 22:	Reporting of Data	Medium	All primer pairs generated specific amplicons which was shown in Fig. 1a. One single peak was obtained in each melting curve analysis, which confirmed the specific amplification of primers (Fig. 1b). The expression of each candidate reference gene showing their Ct values for DEHP is displayed in Fig. 2b. In the results section it was not specifically stated whether the data shown in Fig. 2b was from all timepoints combined and/or whether it was showing a combination of all three treatment concentrations. For DEHP results, there was not data shown for expression levels per treatment concentration or per timepoint sampled.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were adequately described, and the data that was shown included measures of variability.

Additional Comments: This evaluation is for a mechanistic study looking at a reproductive/teratogenic outcome. This study used oocytes from female common carp for the chemical exposure and then extracted cDNA from those exposure samples to run molecular analyses. The goal of this study was to determine the most stable reference gene to assess final oocyte maturation when exposed to DEHP. Based on the results of four different softwares, gapdh was the most stable gene when final oocyte maturation was induced by DEHP. The paper gave adequate details on the molecular analyses ran and the results obtained, but it lacked information regarding test substance preparation and the oocyte DEHP exposure.

Overall Quality Determination N

Medium

Study Citation:	Zhao, X., Ga 23(4):626-63	Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology 23(4):626-632.					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study at	a: 0 - 4 days (0-9 athors (i.e., chen	(6h) nical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vertebrate; F	Fish; Cyprinus carpio; Adult					
Health Outcome:	Mortality Di-ethylbey	vl nhthalate (DEHP)					
HERO ID:	2510817						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2: Metric 3:	Test Substance Purity	Low High	The test substance identity was not analytically verified by the performing laboratory. The chemical grade was reported as analytical			
	Metrie 5.		Ingn	The chemical grade was reported as analyteat.			
Domain 2: Test Design	N / · · /		TT' 1				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5: Matria 6:	Regative Control Response	Low	The biological response of the negative control groups was not reported.			
	Wettie 0.	Kandonnized Anocation	Low	Researchers and not report now organisms were anocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations.			
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported.			
	Metric 9:	Administration Measurement of Test Substance	Low	The exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Low	Spacing was not reported.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	The reported LC50 from this study was 37.9 mg/L for DEHP. The solubility listed in the Final Scope for this compound is 0.27 mg/L.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the choice of the source of the test organisms.			
	Metric 14:	Conditions	Low	The study did not report whether the test organisms were acclimatized.			
	Metric 15:	Number of Organisms and	Low	The numbers of test organisms and replicates were lower than the typical number.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of the environmental conditions was not sufficiently reported to evaluate if they adequate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 2510817 Table: 1 of 1

Study Citation:	Zhao, X. G	ao, Y. Oi, M. (2014). Toxicity of phthalate	esters exposure	to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology		
Study Churchi	23(4):626-63					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-9	6h)		
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Cyprinus carpio; Adult				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2510817					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for outcome assessment were		
		Assessment		reported.		
Demein (. Cenferralia	Warishle Ca					
Domain of Contounding	Matric 10:	IIII01 Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
	Methe 19.	Design and Procedures	Ingn	There were no reported unreferences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
		1	6	66.1		
Domain 7: Data Present	ation and Anal	lysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	The reported	The reported LC50 from this study was 37.9 mg/L for DEHP. The solubility listed in the Final Scope for this compound is 0.27 mg/L.				
Overall Qualit	ty Detern	nination	Medium			

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Study Citation:	Zhao, X., Ga	Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology				
Duration:	23(4):626-63 Overall Dura	23(4):626-632. Dverall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path: Taya Spacios Aga:	Vartabrata: F	ish: Cuprimus carpio: Adult				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	2510817					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 5.	Test Substance Furity	nıgıı	The chemical grade was reported as analytical.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
	Wette 7.	Preparation	Low	the test concentrations.		
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for a dose response.		
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and the spacing of exposure levels were adequate for a		
		Spacing of Exposure Levels	_	dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Low	All treatment concentrations (3.8, 7.59, and 18.9 mg/L) exceeded the solubility of DEHP listed in the final scope at 0.27 mg/L.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the choice of the source of the test organisms.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether the test organisms were acclimatized.		
	Matric 15	Conditions	Low	The numbers of test organisms and replicates were lower than the tunical number		
	Methe 15.	Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.		
Domain 5: Outcome As	sessment		Ŧ			
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of the environmental conditions was not sufficiently reported to evaluate if they were adequate.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 2510817 Table: 1 of 1

		continu	ied from previ	ous page			
Study Citation:	Zhao, X., Ga	Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology					
	23(4):626-63	3(4):626-632.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Fish; Cyprinus carpio; Adult					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2510817						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were reported.			
Demain () Conformation		- 4 1					
Domain 6: Confounding	g / Variable Co	ntroi	TT: -1-				
	Metric 19:	Contounding variables in Test	nign	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quali	ty Deterr	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Chen, X., Xi phthalates ar Overall Dura Aquatic (fres Vertebrate; F Mortality Di-ethylhexy 2298079	u, S., Tan, T., Lee, S. T., Cheng, S. H., Lee nd their mixtures. International Journal of E ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study a Fish; <i>Danio rerio</i> ; AB Strain; Embryo yl phthalate (DEHP)	, F., F.W., Xu, L nvironmental Re n: 0 - 4 days (0-9 uthors (i.e., chen	., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of search and Public Health 11(3):3156-3168. 6h) nical of interest in exposure water, but unable to determine exact uptake route)
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	High High Low	The CAS numbers and structures for BBP, DBP, DEHP, DIDP, and DINP are reported. The sources were listed. The purity/grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a blank control and solvent control were used in the acute bioassays.
	Metric 5:	Negative Control Response	Low	Survival of the controls were not reported.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	Low	Dilution of the test substance into medium was not well described (unclear if embryo
		Preparation		rearing medium was utilized), and the test substance was not renewed over a 72 hr period.
	Metric 8:	Consistency of Exposure	Medium	Exposures appear to have been administered consistently.
	Metric 9:	Administration Measurement of Test Substance	Low	Concentrations are reported as nominal.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	Acute exposures were 72-hr for the embryos, which is slightly shorter than the standard 96-hour acute bioassay.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors reported conducting exposures at 9 concentrations: 500, 100, 50, 10, 1.5, 0.6, 0.3, 0.06, and 0.01 mg/L.
	Metric 12:	Testing at or Below Solubility Limit	Low	Several concentrations were above estimated solubility limits, including 3-4 treatment concentrations (BBP, DBP) or 7 concentrations (DEHP, DIDP, and DINP). Methanol was utilized to improve solubility.
Domain 4: Test Organis	m			
C	Metric 13:	Test Organism Characteristics	High	The source and the strain were reported, and the husbandry methods were adequately described.
	Metric 14:	Acclimatization and Pretreatment	Low	An acclimation period was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each concentration was represented by 20 embryos (1 embryo per well).

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Diethylhexyl Phthalate

		conti	nued from previo	bus page			
Study Citation:	Chen, X., X phthalates a	Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)			
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Vertebrate; H	Vertebrate; Fish; Danio rerio; AB Strain; Embryo					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2298079						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	The 24 well plates were described but lacked details on water conditions (or use of embryo rearing medium) and temperature.			
	Metric 17:	Outcome Assessment Methodology	Low	It was not reported how mortality was determined in the embryos.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment appeared to be consistently conducted across treatment and			
		Assessment		control groups at 72 hr post treatment.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate that animal health or attrition interfered with the bioas- say.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	There were no details on LC50 estimation methods.			
	Metric 22:	Reporting of Data	Low	The authors report LC50 values for BBP and DBP (and LC50 not attained for DEHP, DINP, and DIDP). Mortality was not reported for each treatment group or for the controls.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.			
Additional Comments:	None						
Overall Oualit	tv Deterr	nination	Medium				

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Study Citation:	Kinch, C. D.	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations						
Duration: Exposure Route, Media, Path:	of contamina Overall Dura Aquatic (fres	verall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) quatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa. Species. Age:	Vertebrate: F	Vertebrate: Fish: <i>Danio rerio</i> : Wild Type, long tail: Embryo						
Health Outcome:	Developmen	t/Growth	- J -					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	3350278							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-				
	Weate 7.	Preparation	mgn	scribed in adequate detail.				
	Metric 8:	Consistency of Exposure	High	The exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was tested.				
	Matria 12	Spacing of Exposure Levels	High	Evenesure concentrations ware at an kalaw the water calukility limit				
	Metric 12.	Testing at of Below Solubility Limit	nigii	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organisi	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and	Low	The number of organisms varied. It was unclear if replicates were used				
		Replicates per Group						
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
		Conti	nued on ne	xt page				
Commerce of next Page								

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. Aquatic Toxicology 175:286-298. **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media, Path: Taxa, Species, Age: Vertebrate; Fish; Danio rerio; Wild Type, long tail; Embryo **Health Outcome:** Development/Growth Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 3350278 Domain Metric Rating Comments Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure High There were no differences among groups. Domain 7: Data Presentation and Analysis Statistical Methods Metric 21: High Statistical methods (including any calculations or data transformations) were clearly described. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes. Additional Comments: Results from mixtures were also reported.

Overall Quality Determination

High

Study Citation:	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. Aquatic Toxicology 175:286-298.						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 3350278	Vertebrate; Fish; <i>Danio rerio</i> ; Wild Type, long tail; Embryo Mortality Di-ethylhexyl phthalate (DEHP)					
Domain	0000270	Metric	Rating	Comments			
Domain 1: Test Substar	ice	moune	ruung	Comments			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Cl	naracterization						
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.			
	Metric 8:	Consistency of Exposure	High	The exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	sm						
0.1	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms varied. It was unclear if replicates were used.			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
		Co	ntinued on next page				

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 3350278 Table: 2 of 2

		co	ontinued from previous p	age		
Study Citation:	Kinch, C. D.	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations				
D	of contamina	ants individually and in mixture. Aquatic Tox	ticology 175:286-298.			
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study aut	thors (i.e., chemical of inte	erest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Fish; Danio rerio; Wild Type, long tail; Embr	yo			
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	3350278					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group		
	Metric 23	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	infeatie 25.	Explanation of Chexpected Outcomes	Ingn			
Additional Comments:	Results from mixtures were also reported.					
Overall Qualit	ty Deterr	nination	Uninformative			

Study Citation: Duration: Exposure Route, Media, Path:	Corradetti, F (Danio rerio Overall Dura Aquatic (free	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (Danio rerio). Reproductive Biology 13(3):195-202. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; H Reproductiv Di-ethylhexy	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Di ethylhexyl apthalate (DEHP)					
HERO ID:	2000753	() primi () () ()					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on the exposure administration.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only two levels were tested, but an adequate response was observed.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit. The second concentration, $20 \ \mu g/L$, exceeded the water solubility limit.			
Domain 4: Test Organis	m						
- shan in rost organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were adequate numbers to allow subsampling, but replicates were not used.			
Domain 5: Outcome Ass	sessment						

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Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 2000753 Table: 1 of 2

	continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Corradetti, E (Danio rerio) Overall Dura Aquatic (free Vertebrate: E	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish (Danio rerio). Reproductive Biology 13(3):195-202. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Health Outcome:	Reproductive	e/Teratogenic	e (e.g., fungi of al			
Chemical:	Di-ethylhexy	/l phthalate (DEHP)				
HERO ID:	2000753					
Domain	Metric Rating Comments					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate. Some parameters were reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment					
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quality Determination			Medium			

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HERO ID: 2000753 Table: 2 of 2

Study Citation:	Corradetti, E	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish					
Duration:	Overall Dura). Reproductive Biology 13(3):195-202. ation: 11 - 21 days: Exposure Duration: 11	- 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Fish; Danio rerio; Wild type; Not Applicable	e (e.g., fungi or a	lgae studies) or Not Reported			
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)-Reproductive/Teratogenic					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2000753	2000753					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		Ŧ				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for the assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	aractorization						
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
	Wieure 7.	Preparation	LOW	the test concentrations.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only two levels were tested, but an adequate response was observed.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit. The second concentration, 20 μ g/L, exceeded the water solubility limit.			
Domain 4: Test Organis	m						
2 children in Test Organis.	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were adequate numbers to allow subsampling but replicates were not used.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	The environmental conditions were not sufficiently reported to evaluate if they were adequate. Some parameters were reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
		Conti	nued on next pa	ge			

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HERO ID: 2000753 Table: 2 of 2

		contin	ued from previo	us page		
Study Citation:	Corradetti, E	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish				
	(Danio rerio). Reproductive Biology 13(3):195-202.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	· 21 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chemi	cal of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; Wild type; Not Applicable	e (e.g., fungi or al	gae studies) or Not Reported		
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	aling/function-Ge	enotox (including DNA repair)-Reproductive/Teratogenic		
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2000753					
Domain	Metric Rating Comments					
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
	Assessment					
Domain 6: Confounding	v / Variable Co	ntrol				
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Metric 17.	Design and Procedures	Low	conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain /: Data Present	ation and Anal		TT' 1			
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Medium			

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Study Citation:	Buerger, A.	suerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, I. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Exam- ning the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylbexyl phthalate Environmental Pollution					
	245(Elegricity)	sponses of the zebransh (Danio rerio) ga	strointestinai	system to the suspected obesogen diethylnexyl phinalate. Environmental Pollution			
Duration	Overall Dur	243(Elsevier):1080-1094. Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route	Aquatic (free	Aguatic (freshwater): Food/Diet: Dietary					
Madia Path.	Aquatic (iie)	Aqualic (lieshwaler), Food/Diet, Dietary					
Tava Spacias Aga	Vertebrate: F	Vertebrate: Fich: Dania raria: Wild type (AB strain): Adult					
Health Outcome	Development/Growth						
Chemical:	Di-ethylbey	vl phthalate (DEHP)					
HFRO ID.	5043619	5042610					
Domain	5045017	Matria	Dating	Commente			
Domain Domain 1: Toot Substan		Metric	Kating	Comments			
Domain 1: Test Substan		Trat Salating a Identity	τ				
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemi- cal descriptors were not provided.			
	Metric 2:	Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity was given as "pure." A search on the Sigma Aldrich site for the given stock number shows it was an analytical standard.			
Domain 2: Test Design	Matria 4.	Nanative Controls	II: -1-				
	Metric 4:	Negative Controls	High	A negative control was included.			
	Metric 5:	Negative Control Response	High	The responses of the negative controls were acceptable.			
	Metric 6:	Randomized Allocation	Medium	Randomized allocation was mentioned in the supplementary methods.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media	High	Test media preparation (DEHP stock solution and feed treatment with DEHP) was de-			
		Preparation	U	scribed in detail in the supplemental material and methods.			
	Metric 8:	Consistency of Exposure	High	DEHP was administered in feed and administered consistently across treatment groups;			
		Administration		details were provided in the supplemental methods.			
	Metric 9:	Measurement of Test Substance	High	Concentrations were measured by GC/MS (n=3).			
	Matria 10	Concentration	II: -h				
	Metric 10:	Exposure Duration and Frequency	High	The organisms were red $2x$ daily for 60 days.			
	Metric 11:	Spacing of Exposure Levels	N/A	I ne study only had one dose (5 mg/kg feed) and was not intended to examine a dose-			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via diet			
	wienie 12.	resung at or below Solubility Lillin	11/71				
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).			
	Metric 14:	Acclimatization and Pretreatment	High	Acclimatization for 1 week was noted in the supplemental material, and the pre- treatment culture conditions were the same.			
		Cont	inued on nev	/t nage			

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Environmental Hazard Evaluation

HERO ID: 5043619 Table: 1 of 3

		conti	nued from p	revious page			
Study Citation:	Buerger, A. ining the res 245(Elsevier	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; Wild type (AB strain); Ad	dult				
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5043619						
Domain		Metric	Rating	Comments			
Domain	Metric 15	Number of Organisms and	Medium	There were 6 fish per tank 10 replicate tanks per treatment 60 fish per group, and 30			
	Methe 15.	Replicates per Group	Wedium	tanks total			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal hus- bandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.			
	Metric 17:	Outcome Assessment Methodology	High	Tank mass was measured weekly; individual masses were measured 3x. Gonads and livers were weighed at the end of the experiment to calculate gonadosomatic index (GSI) and hepatosomatic index (HSI).			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across treatments.			
		Assessment					
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods (ANOVA, Kruskal-Wallis tests) were detailed in the supplemental information.			
	Metric 22:	Reporting of Data	High	Data was reported for all groups.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The expected outcome was an increase in weight gain among DEHP-treated fish in the "overfed" category, but there was no difference noted. The given explanation involv- ing genes for lipid metabolism only partially explained this discrepancy, & the authors hypothesized with limited evidence that a longer exposure would lead to the expected outcome.			
Additional Comments:	The objectiv Zebrafish we by weight g gene express hepatosomat	e of this study was to determine how DEHI ere orally exposed to overfeeding and ove ain, body mass index, gonadosomatic ind sion in the intestine (using qPCR and RNS ic index (HSI) under the health outcome of	P in the diet n erfeeding with lex (GSI), ho seq). This for growth/develop	nay exacerbate mechanisms associated with weight gain that occurs with overfeeding. h DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured epatosomatic index (HSI), histological examination of the intestine, and changes in prm was used to evaluate weight gain, body mass index, gonadosomatic index (GSI), elopment.			
Overall Quali	ty Determ	nination	High				

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HERO ID: 5043619 Table: 1 of 3

		continued from previous page				
Study Citation:	Buerger, A. N., Schmidt, J., Chase, A., ining the responses of the zebrafish (Dan 245(Elsevier):1086-1094	Paixao, C., Patel, T. N., Brumback, I io rerio) gastrointestinal system to the	B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Exam- suspected obesogen diethylhexyl phthalate. Environmental Pollution			
Duration:	Overall Duration: > 21 days; Exposure Du	ration: > 21 days				
Exposure Route,	Aquatic (freshwater); Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult					
Health Outcome:	Development/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5043619					
Domain	Metric	Rating	Comments			

Study Citation: Duration: Exposure Route,	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary							
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Gastrointesti Di-ethylhexy 5043619	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult Gastrointestinal Di-ethylhexyl phthalate (DEHP) 5043610						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		0					
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemi- cal descriptors were not provided.				
	Metric 2:	Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.				
	Metric 3:	Test Substance Purity	High	The purity was given as "pure." A search on the Sigma Aldrich site for the given stock number shows it was analytical standard.				
Domain 2: Test Design								
C C	Metric 4:	Negative Controls	High	A negative control was included.				
	Metric 5:	Negative Control Response	High	The response of the negative control was acceptable.				
	Metric 6:	Randomized Allocation	Medium	A randomized allocation method was mentioned in the supplementary methods.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Hıgh	A flow-through system was detailed in the supplementary methods.				
	Metric 8:	Consistency of Exposure Administration	High	The DEHP was administered in the feed; details of the preparation were given in the supplemental methods.				
	Metric 9:	Measurement of Test Substance Concentration	High	The concentration was measured by GC/MS (n=3).				
	Metric 10:	Exposure Duration and Frequency	High	The organisms were fed 2x daily for 60 days.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study only had one dose (3 mg/kg feed), and it was not intended to examine a dose- response effect.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.				
Domain 4: Test Organisi	m							
	Metric 13:	Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).				
	Metric 14:	Acclimatization and Pretreatment	High	An acclimatization period of 1 week was noted in the supplemental material.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The main experimental design included: 6 fish per tank, 10 replicate tanks per treatment, 60 fish per group, and 30 tanks total. Samples for histology included: stained sections of distal intestine from both treatment groups and the control group ($n = 8$ for each treatment).				

Environmental Hazard Evaluation

HERO ID: 5043619 Table: 2 of 3

		contin	nued from p	previous page	
Study Citation: Duration: Exposure Route, Media, Path:	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate; I	Fish; Danio rerio; Wild type (AB strain); A	dult		
Health Outcome: Chemical: HERO ID:	Gastrointestinal Di-ethylhexyl phthalate (DEHP) 5043619				
Domain		Metric	Rating	Comments	
Domain 5: Outcome As	sessment				
Domain 5. Outcome 743	Metric 16:	Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal husbandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.	
	Metric 17:	Outcome Assessment Methodology	High	Gut histology was performed at the end of the experiment.	
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across treatments.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups in environmental conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	Authors reported that stained sections of the distal intestine from both treatment groups and the control group were evaluated for histological alterations. That is, they were histological observations. Statistical analysis was not necessary.	
	Metric 22:	Reporting of Data	High	Data was reported for all groups.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes in histological gut analysis.	
Additional Comments:	Additional Comments: The objective of this study was to determine how DEHP in the diet may exacerbate mechanisms associated with weight gain that occurs with overfeeding. Zebrafish were orally exposed to overfeeding and overfeeding with DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured by weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI), histological examination of the intestine, and changes in gene expression in the intestine (using qPCR and RNAseq). This form was used to evaluate data on the histological examination of the intestine.				
Overall Qualit	ty Detern	nination	High		

Study Citation: Duration: Exposure Route, Madia Path:	Buerger, A. ining the res 245(Elsevier Overall Dura Aquatic (fres	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5043619							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemi- cal descriptors were not provided.				
	Metric 2:	Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.				
	Metric 3:	Test Substance Purity	High	The purity given as "pure." A search on the Sigma Aldrich site for the given stock num- ber shows it was analytical standard.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	A negative control was included.				
	Metric 5:	Negative Control Response	High	The responses of the negative controls were acceptable.				
	Metric 6:	Randomized Allocation	Medium	A randomized allocation method was mentioned in the supplementary methods.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	A flow-through system was detailed in the supplementary methods.				
	Metric 8:	Consistency of Exposure	High	DEHP was administered in the feed; details of the preparation were given in the supple-				
	Metric 9:	Measurement of Test Substance	High	The concentration was measured by GC/MS (n=3).				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The organisms were fed $2x$ daily for 60 days				
	Metric 11:	Number of Exposure Levels	N/A	The study only had one dose (3 mg/kg feed), and it was not intended to examine a dose- response effect				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.				
Domain 4: Test Organis	m							
-	Metric 13:	Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).				
	Metric 14:	Acclimatization and Pretreatment	High	An acclimatization period of 1 week was noted in the supplemental material.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The main experimental design was as follows: 6 fish per tank, 10 replicate tanks per treatment, 60 fish per group, and 30 tanks total. RNAseq samples included: control group $n=4$, overfed group $n = 3$, overfed + DEHP group $n = 5$ selected from multiple tanks within each treatment.				
	Continued on next page							

Environmental Hazard Evaluation

HERO ID: 5043619 Table: 3 of 3

		contin	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media. Path:	Buerger, A. ining the re 245(Elsevier Overall Dur Aquatic (fre	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish (Danio rerio) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environmental Pollution 245(Elsevier):1086-1094. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary					
Taxa, Species, Age:	Vertebrate; I	Fish; Danio rerio; Wild type (AB strain); Ad	dult				
Health Outcome: Chemical: HERO ID:	Mechanistic Di-ethylhexy 5043619	-Cell signaling/function yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal hus- bandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (qPCR of liver and intestine tissues and RNAseq of intestine tissues) were reported and adequate for the intended outcome of interest (gene expression changes related to digestion in the intestine and liver).			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across treatments.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	lvsis					
	Metric 21:	Statistical Methods	High	Statistical methods (ANOVA, Kruskal-Wallis tests) were detailed in the supplemental information.			
	Metric 22:	Reporting of Data	High	The data was reported for all groups.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes in genetic analysis.			
Additional Comments:	ents: The objective of this study was to determine how DEHP in the diet may exacerbate mechanisms associated with weight gain that occurs with overfeeding. Zebrafish were orally exposed to overfeeding and overfeeding with DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured by weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI), histological examination of the intestine, and changes in gene expression in the intestine (using qPCR and RNAseq). This form was used to evaluate data on gene expression.						
Overall Quali	ty Deterr	nination	High				

Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically								
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (fres	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Danio rerio; AB strain; Larvae							
Health Outcome:	Mortality								
Chemical:	Di-ethylhexy	l phthalate (DEHP)							
HERO ID:	5497528								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue num- ber for the chemical. The correct DEHP CASRN is 117-81-7.					
	Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.					
	Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).					
	Metric 5:	Negative Control Response	High	This form was used to evaluate NOT the direct effects of DEHP exposure on male ze- brafish, but the indirect effects of fraternal exposure on the F1 generation larvae survival rate.					
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the mating pairs (8 male zebrafish per group were randomly collected for mating with wild type females), and they reported that 100 fertilized eggs were randomly collected and cultured until 5 dpf.					
Domain 3: Exposure Ch	aracterization								
	Metric /:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.					
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.					
	Metric 9:	Measurement of Test Substance	Low	The test substance was not analytically verified at the beginning, during, or end of the study.					
	Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.					
		Cont	tinued on nex	t page					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Diethylhexyl Phthalate

... continued from previous page Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically **Study Citation:** exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media. Path: Taxa, Species, Age: Vertebrate; Fish; Danio rerio; AB strain; Larvae **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 5497528 Domain Metric Rating Comments Metric 12: Testing at or Below Solubility Limit High The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP. Domain 4: Test Organism Metric 13: Test Organism Characteristics Medium The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture). The fertilized eggs used for the survival rate effects were produced by crossing exposed males with wildtype females. Metric 14: Acclimatization and Pretreatment High Before DEHP exposure, adult male zebrafish were acclimatized in 20 L tanks for 1 week. Conditions Number of Organisms and Metric 15: Low The numbers used were as follows: Parental male fish: 8 male zebrafish from each ex-Replicates per Group perimental group; 3 replicates per group. This form is to evaluate the offspring/F1 survival rate: 100 fertilized eggs were randomly collected per group and cultured until 5 dpf. It is unclear how many parental replicate tanks are represented by the 100 fertilized eggs.

				-585°.
Domain 5: Outcome Assessment				
Me	etric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chi- nese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.
Me	etric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (crossing of DEHP exposed and control male fish with wild-type female fish and collection and culture of fertilized eggs until 5 dpf, taking counts of larvae that survived) were reported and adequate for the outcome of interest (DEHP effects on F1 generation following exposure of parental male fish).
Met	etric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.
		Assessment		
Domain 6: Confounding / Variable Control				
Met	etric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.
Met	etric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

Continued on next page ...
Diethylhexyl Phthalate

		contin	ued from p	previous page		
Study Citation: Duration: Exposure Route, Media Path:	Ma, Y. B., J exposed to e Overall Dura Aquatic (free	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; AB strain; Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5497528					
Domain		Metric	Rating	Comments		
	Metric 21:	Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean \pm standard error (SEM).		
	Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figure 1B).		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentra- tions by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, and DNA methylation (global and site-specific) and mRNA changes of genes involved in reproduction. This form was used to evaluate the data reported for the F1 generation larvae (offspring growth/development): survival rate.					

Overall Quality Determination

High

Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.				
Duration:	Overall Duration: > 21 days; Exposure Duration	on: > 21 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined b	y study authors (i.e., chemical of	of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Larva	e			
Health Outcome:	Development/Growth				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	5497528				
Domain	Metric	Rating	Comments		
Domain 1: Test Substan	ice				
		T mi i			

Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue num- ber for the chemical. The correct DEHP CASRN is 117-81-7.
	Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control was used $(0.001\% (v/v) DMSO)$.
	Metric 5:	Negative Control Response	High	This form was used to evaluate NOT the direct effects of DEHP exposure on male ze- brafish, but the indirect effects of fraternal exposure on the F1 generation larvae (off- spring growth/development): hatching rate and body weight.
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the mating pairs (8 male zebrafish per group were randomly collected for mating with wild type females), and they reported that 100 fertilized eggs were randomly collected and cultured until 5 dpf.
Domain 3: Exposure Ch	aracterization			
·	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
	Metric 9:	Measurement of Test Substance	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
	Metric 10.	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type
	Metric 11	Number of Exposure Groups/	High	Exposure groups and spacing (control (0.001% DMSO) 10.33 and 100 µg/L DEHP)
	moule II.	Spacing of Exposure Levels		were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentra- tions below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.

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Diethylhexyl Phthalate

Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylbexyl) phthalate. Environmental Pollution 237:1050-1061					
Duration: Exposure Route, Media. Path:	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; AB strain; Larvae				
Health Outcome:	Developmen	t/Growth				
HERO ID:	5497528	/I phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the au- thors did not explicitly clarify the source (e.g., laboratory culture). The fertilized eggs used for the growth/development effects were produced by crossing exposed males with wild-type females.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, adult male zebrafish were acclimatized in 20 L tanks for 1 week.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers were reported as follows: Parental male fish: 8 male zebrafish from each experimental group; 3 replicates per group. This form is to evaluate the offspring/F1 data on growth/development: 100 fertilized eggs were randomly collected per group and cultured until 5 dpf. It is unclear how many parental replicate tanks are represented by the 100 fertilized eggs.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chi- nese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (crossing of DEHP exposed and control male fish with wild-type female fish and collection and culture of fertilized eggs until 5 dpf, counting of hatched larvae, and weighing larvae) were reported and adequate for the outcome of interest (DEHP effects on F1 generation following exposure of parental male fish).		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.		
Domain 6: Confounding	/ Variable Co	ntrol				
Bomani O. Comounding	Metric 19:	Confounding Variables in Test	High	Based on information reported by authors, there were no limitations that would result in		
	M + 1 - 20	Design and Procedures		a substantial impact on results.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean \pm standard error (SEM).		

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Environmental Hazard Evaluation

HERO ID: 5497528 Table: 2 of 5

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Study Citation:	Ma, Y. B., J exposed to e	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Larvae				
Health Outcome:	Developmen	t/Growth			
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	5497528				
Domain		Metric	Rating	Comments	
	Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figure 1B).	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	The goal of t tions by exam methylation	his study was to investigate the chronic imp mining the effect of DEHP on male reprodu (global and site-specific) and mRNA chang	pacts of a 3- uctive capal ges of genes	month DEHP exposure on male reproduction with environmentally relevant concentra- bilities, offspring growth/development, plasma reproductive hormone levels, and DNA s involved in reproduction. This form was used to evaluate the data reported for the F1	

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Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult					
Duration:						
Exposure Route,						
Media, Path:						
Taxa, Species, Age:						
Health Outcome:	Mechanistic	-Cell signaling/function-Epigenetics-Rece	ptor binding/	regulation of receptor activity		
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5497528					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; instead, this number is the cata- logue number for the chemical. The correct DEHP CASRN is 117-81-7.		
	Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.		
Domain 2: Test Design	34.5.4					
	Metric 4:	Negative Controls	High	A solvent control was used $(0.001\% (v/v) DMSO)$.		
	Metric 5:	Negative Control Response	High	DNA methylation (global and site-specific) and gene expression (cyp1/a1, hsd1/b3 and cyp19a1a) in male gonads were assessed. The biological responses of the solvent control were reported and adequate (Figures 5, 6, & 7).		
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the various health outcomes evaluated in this form (sections 2.7 & 2.9).		
Demain 2: Error Ch						
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared		
	Metric 8:	Consistency of Exposure	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups		
	Metric 9:	Auministration Measurement of Test Substance	Low	The test substance was not analytically verified at the beginning, during, or end of the study.		
	Metric 10.	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP)		
		Spacing of Exposure Levels		were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.		
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentra- tions below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.		

Domain 4: Test Organism

Environmental Hazard Evaluation

HERO ID: 5497528 Table: 3 of 5

continued from previous page							
Study Citation:	Ma, Y. B., J	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically					
Duration	Overall Dur	exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.					
Exposure Route	Aquatic (free	shwater): Water: Not determined by study	v authors (i e	chemical of interest in exposure water, but unable to determine exact untake route)			
Media, Path:	riquite (iie	sitvater), water, i tot determined by stad	, uutiloits (i.e.,				
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; AB strain; Adult					
Health Outcome:	Mechanistic	Mechanistic-Cell signaling/function-Epigenetics-Receptor binding/ regulation of receptor activity					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5497528						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the au- thors did not explicitly clarify the source (e.g., laboratory culture).			
	Metric 14:	Acclimatization and Pretreatment	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers were reported as follows: Study Total: 16 male fish per tank, 3 replicates per group, and 4 treatment groups (0, 10, 33, and 100 mg/L DEHP) = 48 male fish per group = 192 male fish total. DNA methylation (global and site-specific) and gene expression (mRNA changes in cyp17a1, cyp19a1a, and hsd17b3): 2 fish per tank were randomly collected as one replicate, and there were 3 replicates per group.			
Domain 5: Outcome As	ssessment Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chi- nese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed			

			according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (DNA methylation (global and site-specific) and gene expression (mRNA changes in cyp17a1, cyp19a1a, and hsd17b3)) were reported and adequate for the outcome of interest (DEHP effects on DNA methylation and genes involved in reproductive capabilities).
Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Con	ntrol		
Metric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Anal	ysis		
Metric 21:	Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean \pm standard error (SEM).
Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figures 5, 6, & 7).
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Environmental Hazard Evaluation

HERO ID: 5497528 Table: 3 of 5

	•••	continued from previous p	nge		
Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.				
Duration:	Overall Duration: > 21 days; Exposure Duration:	> 21 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by s	tudy authors (i.e., chemical of	of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Adult				
Health Outcome:	Mechanistic-Cell signaling/function-Epigenetics-Receptor binding/ regulation of receptor activity				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	5497528				
Domain	Metric	Rating	Comments		
Additional Comments:	The goal of this study was to investigate the chro trations by examining the effect of DEHP on ma methylation (global and site-specific), and mRNA data: DNA methylation (global and site-specific)	nic impacts of a 3-month D le reproductive capabilities, A changes of genes involved and gene expression of cyp1	EHP exposure on male reproduction with environmentally relevant concen- offspring growth/development, plasma reproductive hormone levels, DNA in reproduction. This form was used to evaluate the following mechanistic 7a1, cyp19a1a, and hsd17b3.		
Overall Qualit	ty Determination	High			

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, Y. B., J exposed to e Overall Dura Aquatic (fres Vertebrate; F Mechanistic- Di-ethylhexy 5497528	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult Mechanistic-Biomarkers (exposure and effect) Di-ethylhexyl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	се					
	Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; instead, this number is the cata- logue number for the chemical. The correct DEHP CASRN is 117-81-7.		
	Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).		
	Metric 5:	Negative Control Response	High	Plasma reproductive hormone levels (testosterone and 17beta-estradiol) were assessed. The biological responses of the solvent control were reported and adequate.		
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the outcome pertaining to this form (reproductive hormones, sections 2.5).		
Domain 3: Exposure Ch	aracterization					
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.		
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.		
	Metric 9:	Measurement of Test Substance	Low	The test substance was not analytically verified at the beginning, during, or end of the study.		
	Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study, (Wang et al 2013) as well as being environmentally relevant.		
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentra- tions below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.		

Domain 4: Test Organism

HERO ID: 5497528 Table: 4 of 5

		C	ontinued from p	revious page		
Study Citation:	Ma, Y. B., J exposed to e	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; AB strain; Adult				
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5497528					
Domain		Metric	Rating	Comments		
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the au-		

Domain			i tating	Comments
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the au- thors did not explicitly clarify the source (e.g., laboratory culture).
	Metric 14:	Acclimatization and Pretreatment	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers and replicates are as follows: Study Total: 16 male fish per tank, 3 repli- cates per group, and 4 treatment groups $(0, 10, 33, and 100 \text{ mg/L DEHP}) = 48$ male fish per group = 192 male fish total. Reproductive hormone levels (testosterone and 17beta- estradiol): 4 fish per tank wereconsidered as one replicate. There were 3 replicates per group.
Domain 5: Outcome A	Assessment			
	Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chi- nese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (collection of blood, extraction of plasma, and use of the competitive ELISA) were reported and adequate for the outcome of interest (DEHP effects on the concentration of reproductive hormones and its reproductive out- comes).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confoundi	ng / Variable Co	ntrol		
Domain 0. Comound	Metric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
	1 . 1			
Domain /: Data Prese	ntation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean \pm standard error (SEM).
	Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figure 2).
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Environmental Hazard Evaluation

	conti	inued from previous p	age				
Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., exposed to environmentally relevant concentrations of	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylbexyl) phthalate. Environmental Pollution 237:1050-1061					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 2	1 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study	authors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Adult						
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5497528						
Domain	Metric	Rating	Comments				
Additional Comments:	The goal of this study was to investigate the chronic i trations by examining the effect of DEHP on male re methylation (global and site-specific), and mRNA cha data: plasma reproductive hormone levels (testosterone	mpacts of a 3-month I productive capabilities nges of genes involved e and 17beta-estradiol)	EHP exposure on male reproduction with environmentally relevant concen- , offspring growth/development, plasma reproductive hormone levels, DNA l in reproduction. This form was used to evaluate the following mechanistic				
Overall Qualit	ty Determination	High					

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Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	5497528

Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue num- ber for the chemical. The correct DEHP CASRN is 117-81-7.
	Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).
	Metric 5:	Negative Control Response	High	Fecundity, fertilization success, and histological examination of male gonads were as- sessed. The biological responses of the solvent control were reported and adequate (Figures 1A, 3, 4, & S1).
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the various health outcomes pertaining to this form (sections 2.4, 2.6, & 2.7).
Domain 3. Exposure Ch	aracterization			
Domain J. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
	Metric 8:	Consistency of Exposure	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
	Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentra- tions below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.

Domain 4: Test Organism

HERO ID: 5497528 Table: 5 of 5

		continued from previous page	
Study Citation:	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., exposed to environmentally relevant concent	Lu, C. J., Pei, D. S. (2018). Reprodu rations of di-(2-ethylhexyl) phthalate.	ctive effects linked to DNA methylation in male zebrafish chronically Environmental Pollution 237:1050-1061.
Duration:	Overall Duration: > 21 days; Exposure Dura	ation: > 21 days	
Exposure Route,	Aquatic (freshwater); Water; Not determined	by study authors (i.e., chemical of int	erest in exposure water, but unable to determine exact uptake route)
Media, Path:			
Taxa, Species, Age:	Vertebrate; Fish; Danio rerio; AB strain; Ad	ult	
Health Outcome:	Reproductive/Teratogenic		
Chemical:	Di-ethylhexyl phthalate (DEHP)		
HERO ID:	5497528		
Domain	Metric	Rating	Comments

Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture).			
	Metric 14:	Acclimatization and Pretreatment	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers and the replicates were reported as follows: Study Total: 16 male fish per tank, 3 replicates per group, and 4 treatment groups (0, 10, 33, and 100 mg/L DEHP) = 48 male fish per group = 192 male fish total. Reproductive capabilities (fe- cundity and fertilization success): 8 male zebrafish from each experimental group. Light microscopy: 9 fish randomly collected from three tanks in each group. Electron mi- croscopy: 3 fish randomly collected from 3 tanks in control and 100 mg/L DEHPexpo- sure groups.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chi- nese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies [fecundity (number of eggs produced per fe- male), fertilization success (number of fertilized eggs), light and electron microscopic examination of gonads] were reported and adequate for the outcome of interest (DEHP effects on reproduction and gonad integrity).			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.			
Domain 6: Confounding	o / Variable Cou	atrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain /: Data Present	Mate ¹ - 21	ysis Statistical Mathed	TT: -1-	From the fortilization many and considered in the Control of the			
	Metric 21:	Statistical Methods	Hign	Fecundity, fertilization success, and gonad staging by microscopy: Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values $<$ 0.05 were considered statistically significant. All data are shown as the mean \pm standard error (SEM).			
	Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figures 1A, 3, 4, & S1).			
	Continued on next page						

HERO ID: 5497528 Table: 5 of 5

		contin	ued from	previous page		
Study Citation:	Ma, Y. B., Jia, P. exposed to enviror	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylbexyl) phthalate. Environmental Pollution 237:1050-1061.				
Duration:	Overall Duration:	> 21 days; Exposure Duration: $>$ 21	days			
Exposure Route,	Aquatic (freshwate	er); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; L	Danio rerio; AB strain; Adult				
Health Outcome:	Reproductive/Tera	togenic				
Chemical:	Di-ethylhexyl phtl	nalate (DEHP)				
HERO ID:	5497528					
Domain		Metric	Rating	Comments		
	Metric 23: Exp	planation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	: The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentra- tions by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, and DNA methylation (global and site-specific) and mRNA changes of genes involved in reproduction. This form was used to evaluate the data reported for male reproductive effects: Fecundity, fertilization success, and histological examination of male gonads (light and electron microscopy).					
Overall Qualit	y Determina	ation	High			

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 3071151					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not re- ported.		
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).		
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.		
Domain 2: Test Design	Metric 4:	Negative Controls	N/A	Progeny from previously exposed parents were reared, so these F1 fish were not directly		
	Metric 5:	Negative Control Response	N/A	exposed. F1 progeny from control F0 parents were represented. Progeny from previously exposed		
	Metric 6:	Randomized Allocation	Medium	Random allocation of larvae was noted in section 2.5.		
Domain 3: Exposure Cha	aracterization					
-	Metric 7:	Experimental System/Test Media Preparation	Low	The rearing of fish was presented in limited detail.		
	Metric 8:	Consistency of Exposure	High	It appears the treatment and control groups were handled the same.		
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	These F1 larvae were not exposed to the chemical, but outcomes resulted from parents that had chronic exposures.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.		
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.		
Domain 4: Test Organist	n					
	Metric 13:	Test Organism Characteristics	High	Source of these F1 fish were from previously exposed parents within the current experi- ment.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 3071151 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.						
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (1.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Embryo						
Health Outcome:	Developmen	nt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	30/1151						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Values presented as mean Hatch rate, malformation rate, and survival were represented by three replicates of 100 embryos/larvae. Mean body weight is represented by triplicate groups with 20 larvae per replicate.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Housing from hatch to 7 dph was not well described.			
	Metric 17:	Outcome Assessment Methodology	Low	Body weight of F1 larvae from the highest treatment concentration (F0 parents) was			
				significantly lower than the control (Table 3). Methods for acquiring larval weight were not described. Criteria for malformation rate were not described.			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to suggest animal attrition or health impacted results.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.			
	Metric 22:	Reporting of Data	High	Mean and SEM for output parameters are reported in table 3 on page 3/7.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Ouali	tv Deterr	nination	High				

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Study Citation:	Guo, Y., Yan	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocumic rarue). Environmental Pollution 203(Elequicr):130-136					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 2	1 days	э.			
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate: F	Vertebrate: Fish: Gobiocypris rarus: Adult					
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	e M (1		т				
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly. CAS and chemical structure were not re- ported.			
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).			
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.			
Domain 2: Test Design							
8	Metric 4:	Negative Controls	High	Solvent controls were used at a DMSO concentration of 0.005%.			
	Metric 5:	Negative Control Response	High	Control metrics for reproduction (egg production per day per fish) and Gondanosomatic index were reported on Table 2 and Figure 1 (page 3/7).			
	Metric 6:	Randomized Allocation	Medium	The random allocation of larvae in treatment groups at the beginning of the exposure was listed in section 2.2.			
Domain 3: Exposure Cha	aracterization						
1	Metric 7:	Experimental System/Test Media Preparation	High	The experimental systems were detailed in 2.2. The fish were moved to different units as they grew from hatch to 6 months post hatch for the 4 week spawning experiment.			
	Metric 8:	Consistency of Exposure	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treat- ment and control groups were handled the same			
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	Exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawn- ing at 6 months post hatch.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.			
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.			
Domain 4: Test Organist	n						
U	Metric 13:	Test Organism Characteristics	Low	The source was not listed.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).			
Continued on next page							

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Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 3071151 Table: 1 of 5

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Study Citation:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarue). Environmental Pollution 203(Elsavier):130, 136						
Duration:	Overall Duration: > 21 days: Exposure Duration: > 21 days						
Exposure Route.	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate: Fish: Gobiocypris rarus; Adult						
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	/l phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 2).			
Domain 5: Outcome As	sessment	Reproducts per Group					
Domain 5. Outcome rist	Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of containers but the authors did not report water quality parameters throughout the long-term exposure study, and they did not report the density (g/L) of the fish in each replicate.			
	Metric 17:	Outcome Assessment Methodology	High	Reproductive output was significantly lower at the two highest concentrations (figure 1) and GSI in females was significantly lower than control for the highest treatment concentration (table 2).			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.			
Domain 6: Confounding	r / Variable Cou	ntrol					
Domain 0. Comounding	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in the study to suggest animal attrition or health impacted results.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.			
	Metric 22:	Reporting of Data	High	Male and female GSI are presented as means with standard error of the mean (table 2) and egg output is also presented with SEM (figure 1).			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Qualit	v Detern	nination	High				

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HERO ID: 3071151 Table: 2 of 5

Study Citation:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypric rarus). Environmental Pollution 203(Elegvier):130-136						
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Developmen Di-ethylhexy 3071151	Fish; <i>Gobiocypris rarus</i> ; Adult t/Growth /l phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not re- ported.			
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).			
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Solvent controls were used at a DMSO concentration of 0.005%.			
	Metric 5:	Negative Control Response	High	Control metrics for growth (length, weight, and condition factor) are reported in Table 2 (page 3/7).			
	Metric 6:	Randomized Allocation	Medium	The random allocation of larvae in treatment groups at the beginning of exposure was listed in section 2.2.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	The experimental systems were detailed in 2.2. The fish were moved to different units as			
	incure /.	Preparation	mgn	they grew from hatch to 6 months post hatch for the 4 week spawning experiment.			
	Metric 8:	Consistency of Exposure Administration	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treat- ment and control groups were handled the same.			
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	Exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawn- ing at 6 months post hatch.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.			
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.			
Domain 4: Test Organis	m						
rest organis	Metric 13:	Test Organism Characteristics	Low	The source was not listed.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 2).			

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Environmental Hazard Evaluation

HERO ID: 3071151 Table: 2 of 5

		conti	nued from p	revious page			
Study Citation:	Guo, Y., Yar (Gobiocypri	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Gobiocypris rarus; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of containers, but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.			
	Metric 17:	Outcome Assessment Methodology	Low	Length, weight, and condition factor of the F0 adults were not significantly different from the control after 7 months exposure to DEHP at concentrations of 4.2, 13.3, and 40.8 in females. There were inconsistent differences in male weight and condition factor at the middle treatment concentration when compared to the control (Table 2).			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.			
Domain 6: Confounding	g / Variable Co	ntrol					
2011111 01 20110011011	Metric 19:	Confounding Variables in Test	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted the results.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.			
	Metric 22:	Reporting of Data	High	Length, weight and condition factors for males and females are presented as means with standard error of the mean (table 2).			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	This evaluat	on includes weight, length, GSI, and HSI					
Overall Quali	ty Detern	nination	High				

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HERO ID: 3071151 Table: 3 of 5

Study Citation:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow						
Duration:	Overall Dur	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path: Taya Species Age:	Vertebrate: I	Fish: Gobiocopris rarus: Adult					
Health Outcome	Mechanistic	-Cell signaling/function-Endocrine toxicity	I				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	— • • • •	-				
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not re- ported.			
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).			
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Controls were used for the gene expression portion of this investigation.			
	Metric 5:	Negative Control Response	High	Controls were used in this gene expression group, and the gene responses are presented in Table 4 (page 5/7).			
	Metric 6:	Randomized Allocation	Medium	The random allocation of larvae in treatment groups at the beginning of exposure was listed in section 2.2.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental systems were detailed in 2.2. The fish were moved to different units as they grew from hatch to 6 months post hatch for the 4 week spawning experiment.			
	Metric 8:	Consistency of Exposure Administration	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treat- ment and control groups were handled the same.			
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.			
	Metric 10:	Exposure Duration and Frequency	Medium	Exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawn- ing at 6 months post hatch. This is a snapshot of gene expression at the end of a spawn- ing period.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.			
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.			
Domain 4: Test Organis	m						
2 onium 1. rost organis	Metric 13:	Test Organism Characteristics	Low	The source was not listed.			
	Metric 14:	Acclimatization and Pretreatment	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There are three replicates with only 2 fish per replicate (Table 4).			
		Cont	inued on nex	ct page			

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Environmental Hazard Evaluation

HERO ID: 3071151 Table: 3 of 5

		conti	nueu from p	revious page			
Study Citation:	Guo, Y., Yar (Gobiocypris	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Gobiocypris rarus; Adult					
Health Outcome:	Mechanistic	-Cell signaling/function-Endocrine toxicity	7				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of containers, but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.			
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment for gene expression was well detailed and used a housekeeping gene to normalize background expression via the delta delta CT method. Organs for each gene primer were targeted (table 4).			
	Metric 18:	Consistency of Outcome	High	The assessment protocol appears to be assessed consistently among the treatment groups			
		Assessment		and control.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted results.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.			
	Metric 22:	Reporting of Data	High	Mean gene transcription and standard error of the mean (table 4) were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	This evaluati	on includes gene transcription and hormor	ne concentrat	ions.			

Overall Quality Determination

High

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HERO ID: 3071151 Table: 4 of 5

Study Citation: Duration: Exposure Route, Media. Path:	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnov (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 3071151	Fish; <i>Gobiocypris rarus</i> ; Adult /l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not re- ported.		
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).		
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Controls were used for the hepatosomatic index (HSI) portion of this investigation.		
	Metric 5:	Negative Control Response	High	Control HSI values for males and females are presented in table 2 (page 3/7).		
	Metric 6:	Randomized Allocation	Medium	The random allocation of larvae in treatment groups at the beginning of exposure was listed in section 2.2.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental systems were detailed in 2.2. The fish were moved to different units as they grew from hatch to 6 months post hatch for the 4 week spawning experiment.		
	Metric 8:	Consistency of Exposure Administration	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treat- ment and control groups were handled the same.		
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.		
	Metric 10:	Exposure Duration and Frequency	Medium	The exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawning at 6 months post hatch. This is a snapshot of gene expression at the end of a spawning period.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.		
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.		
Domain 4: Test Organisi	m					
0	Metric 13:	Test Organism Characteristics	Low	The source was not listed.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 4).		

Diethylhexyl Phthalate

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Study Citation:	Guo, Y., Yan (Gobiocypris	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	ish; Gobiocypris rarus; Adult				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	3071151					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of containers. but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.		
	Metric 17:	Outcome Assessment Methodology	High	Mortality was reported in Table 3.		
	Metric 18:	Consistency of Outcome	High	The assessment protocol appears to be assessed consistently among the treatment groups		
		Assessment				
Domain 6: Confounding	g / Variable Coi	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted results.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.		
	Metric 22:	Reporting of Data	High	Mean HSI and standard error of the mean (table 2) were presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments: This form is for mortality with results presented in Table 3 and in section 3.2 in the text.						

Overall Quality Determination

High

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Study Citation:	Guo, Y., Yar	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow						
Duration:	(Gobiocypris	s rarus). Environmental Pollution 203(Elsevie ation: > 21 days: Exposure Duration: > 21 d	er):130-136.					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study aut	hors (i.e., chemical of i	nterest in exposure water, but unable to determine exact uptake route)				
Media, Path:	-							
Taxa, Species, Age:	Vertebrate; F	Fish; Gobiocypris rarus; Adult						
Chemical:	Di-ethylhexy	r vl nhthalate (DEHP)						
HERO ID:	3071151							
Domain		Metric Rating Comments						
Domain 1: Test Substan	ce		T					
	Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not re- ported.				
	Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).				
	Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Controls were used for the hepatosomatic index (HSI) portion of this investigation.				
	Metric 5:	Negative Control Response	High	Control HSI values for males and females are presented in table 2 (page 3/7).				
	Metric 6:	Randomized Allocation	Medium	Random allocation of larvae in treatment groups at the beginning of exposure was reported in section 2.2.				
Domain 3: Exposure Ch	aracterization							
Ĩ	Metric 7:	Experimental System/Test Media Preparation	High	The experimental systems were detailed in 2.2. The fish were moved to different units as they grew from hatch to 6 months post hatch for the 4 week spawning experiment.				
	Metric 8:	Consistency of Exposure Administration	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treat- ment and control groups were handled the same.				
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.				
	Metric 10:	Exposure Duration and Frequency	Medium	Exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawn- ing at 6 months post hatch. This is a snapshot of gene expression at the end of a spawn- ing period.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.				
	Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source was not listed.				
	Metric 14:	Acclimatization and Pretreatment	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 4).				

Diethylhexyl Phthalate

		co	ntinued from previous	page			
Study Citation:	Guo, Y., Yar (Gobiocypris Overall Dura	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow (Gobiocypris rarus). Environmental Pollution 203(Elsevier):130-136. Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater): Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	1						
Taxa, Species, Age:	Vertebrate; F	Fish; Gobiocypris rarus; Adult					
Health Outcome:	Hepatic/Live	er					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	3071151						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of the containers, but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.			
	Metric 17:	Outcome Assessment Methodology	Uninformative	The study found no differences in HSI among treatment concentrations and the control (Table 2).			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and the control.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted results.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.			
	Metric 22:	Reporting of Data	High	Mean HSI and standard error of the mean (table 2) were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			

Additional Comments: This criteria is unacceptable because no differences in HSI among treatment concentrations and control were found (Table 2).

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.							
	Environmen	tal Pollution 27(4):263-274.						
Duration: Exposure Poute	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	ays thors (i.e., chemical of in	starest in exposure water, but unable to determine exact untake route)				
Media, Path:	Aquatic (IIe	require (neshwater), water, not determined by study authors (ne., enermear of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Lampetra planeri; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (bio	transformation)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	59542							
Domain		Metric Rating Comments						
Domain 1: Test Substance	ce Matria 1.	Test Substance Identity	Madian					
	Metric 1:	Test Substance Identity	Medium	CASRN or structure were reported.				
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.				
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.				
Domain 2: Test Design	Matria 4.	Na antina Caratura la	TT					
	Metric 4: Matria 5:	Negative Controls	Uninformative	No negative control group was reported.				
	Metric 5:	Randomized Allocation	IN/A Low	No negative control group was reported.				
	Metric 0.	Kandonnized Anocation	LOw	No fandom anocation was reported.				
Domain 3: Exposure Cha	aracterization							
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media	Medium	Biomass loading and placement of organisms in 20L tank was not reported.				
		Preparation						
	Metric 8:	Consistency of Exposure	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP.				
	Metric 0.	Administration Measurement of Test Substance	High	DEHP was dissolved in accione prior to adding to system. The test substance was measured by TLC and liquid scintillation. Final concentrations				
	Metric 9.	Concentration	Ingn	of sediment, glass walls, surface microlaver, suspended material, and groups of organ-				
		concentration		isms were completed at the end of the study. Mass balances were calculated. The mass				
				balance was DEHP + metabolites (phthalic acid and phthalic anhydride).				
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.				
	Metric 11:	Number of Exposure Groups/	N/A	Only one exposure concentration was utilized in this study.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than its approximate sol-				
	Methe 12.	Testing at of Delow Solubility Linit	Wiedrum	ubility in water (0.3 mg/L). Use of acetone as a solvent may increase the solubility				
				slightly.				
Domain 4: Test Organisr	Metric 13	Test Organism Characteristics	Low	Organisms ware collected in the field. Age or say was not provided in the study				
	Metric 14	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment				
		Conditions	2011	The study did not report doomination of prodoutinent.				
	Metric 15:	Number of Organisms and	Low	No replicates were reported.				
		Replicates per Group						

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page				
Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Lampetra planeri; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biot	transformation)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	59542							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of feed for fish was not well described.				
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in the tissue of organisms as well as in the water and sediment were described, but details of the organism preparation for extraction was not described (euthanasia, if applicable, or harvesting of plant material).				
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms were loaded into same experimental tank which could affect 14C DEHP uptake by each organism.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.				
Domain 7: Data Presentation and Analysis								
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.				
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.				
Additional Comments:	This study of mesocosm. (may not be a	Metric 23: Explanation of Unexpected Outcomes Low No variability was reported. This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured						

Overall Quality Determination

Uninformative

DEHP + metabolites, the metabolites being phthalic acid and phthalic anhydride.

Study Citation:	Adams, W. J organisms, H	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.						
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome:	Vertebrate; F Mortality	Fish; Lepomis macrochirus; Juvenile						
HERO ID:	1321996	yi pitilalate (DEHF)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		Ŧ					
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.				
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.				
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5:	Negative Control Response	High	The control response was acceptable.				
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.				
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of exposure were appropriate for the test.				
	Metric 11:	Number of Exposure Groups/	High	The exposure levels were appropriate. A range finding test was performed.				
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.				
Domain 5: Outcome Ass	sessment	· · ·						

	continued from previous page					
Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic		
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-1	574.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Lepomis macrochirus; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.		
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.		
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent across groups.		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The environmental conditions were consistent across groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	The statistical methods were performed and described.		
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					
Overall Quality Determination		High				

Study Citation: Duration: Exposure Route, Media, Path:	Bionomics, (Overall Dura Aquatic (free	Bionomics, (1982). Bioassay report acute toxicity of compounds to bluegill (Lepomis macrochirus). Prepared by Bionomics Inc with cover letter. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 1316181	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN on the title page.				
	Metric 2:	Test Substance Source	Low	The source of the DEHP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity of the DEHP was not reported.				
Domain 2: Test Design								
Domain 21 Test Design	Metric 4:	Negative Controls	High	Table 5 indicates the use of a concurrent negative control.				
	Metric 5:	Negative Control Response	High	The negative control response is reported n Table 5 and is appropriate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the bluegills were allocated into study groups.				
Domain 3: Exposure Cl	naracterization							
-	Metric 7:	Experimental System/Test Media Preparation	Low	The system was reported to be a static system. Little other details were provided on the test system. Details regarding the preparation of the test concentrations were limited.				
	Metric 8:	Consistency of Exposure Administration	Low	All tests were conducted in 5 gallon glass vessels kept in water baths at 18C. Little other information was provided on test administration. Test volume, number of organisms per test chamber, and measured test concentrations were not reported.				
	Metric 9:	Measurement of Test Substance Concentration	Low	Samples taken for the analytical measurements of DEHP are reflected in Table 3. How- ever, important relevant details regarding these measurements are missing.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of the test was 96h, which is appropriate for an acute toxicity test with bluegills.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups, and the spacing was adequate, though no response was observed.				
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	Test concentrations were above the water solubility limit of DEHP. TL50 values were based off nominal values.				
Domain 4: Test Organis	sm							
	Metric 13:	Test Organism Characteristics	Medium	The bluegills were reported to be from a commercial fishery in Nebraska, but the actual name of it was not reported. The age of the fish was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	The bluegills were reported to be acclimated for at least 24h prior to the start of the test.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and the number of replicates were not reported.				
Domain 5: Outcome As	sessment							

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1316181 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Bionomics, (1982). Bioassay report acute toxicity of compounds to bluegill (Lepomis macrochirus). Prepared by Bionomics Inc with cover letter. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1316181						
Domain	Metric Rating Comments						
	Metric 16:	Adequacy of Test Conditions	Low	Little details regarding the environmental conditions of the organisms were provided.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-mortality expressed as TL50 values.			
	Metric 18:	Consistency of Outcome Assessment	High	Test organisms were assessed for mortality at 24 and 96 hours in the study.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Little information was provided on the test conditions of the organisms, and comparison could not be made.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	TL50 values were determined by converting test concentrations and the corresponding observed percent mortalities to logs and probits. These were used to calculate a linear regression equation.			
	Metric 22:	Reporting of Data	High	Control and exposure responses for mortality are reported in Table 5.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	No unexpected outcomes were reported, but confidence intervals were unable to be calculated because no mortalities were reported at any of the test concentrations. From the other chemical tested, it did not look like variability was reported in the percent mortality.			
Additional Comments:	This study v received an u	vas on the acute toxicity of DEHP to bluegill inacceptable rating because all test concentration	fish. Mortality was to ons appeared to be ab	he outcome of interest expressed in the form of TL50 values. The study ove the water solubility limit of DEHP. Study authors did not report mean			

measured values. Only nominal concentrations were reported, and TL50 values were based off of the nominal values.

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome:	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1316201					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance- Diisooctylphthalate (isomer of DEHP) was identified by chemical name and CASRN (27554-26-3).		
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design	Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of test substance before and during the expo- sure. Measured concentrations deviated from reported nominal concentrations		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at the initiation and termination of the experi- ment. Measured concentrations deviated from nominal concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type- 96 hour acute toxicity test.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response by the study authors and adequate to address the purpose of the study. For Di- isooctylphthalate (isomer of DEHP), a preliminary test was conducted, which indicated that it was not toxic below the water solubility limit. A corroborative test was then con- ducted exposing the bluegill to a single replicated concentration of Diisooctylphthalate (isomer of DEHP) representing its limit of water solubility.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at the water solubility limit.		
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.		
Continued on next page						

Diethylhexyl Phthalate

HERO ID: 1316201 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1316201						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 blue gill in each test jar, and they were tested in duplicates.			
Domain 5: Outcome As	Domain 5: Outcome Assessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
Domain 6: Confounding	. / Variable Co	ntrol					
Domain 0. Comounding	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	veic					
Domain 7. Data i resent	Metric 21:	Statistical Methods	N/A	There was no mortality at the concentration tested. Therefore, statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group. Negative findings were reported quantitatively (Table 4).			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Non-definitive LC 50 values reported based on a corroborative test (following negative findings from a preliminary test at concentration below the limit of water solubility) conducted by exposing bluegills to a single replicated concentration of Diisooctylphthalate (isomer of DEHP) representing its limit of water solubility.Diisooctylphthalate CASN 27554-26-3 is listed as a relevant form on the PECO within the Final scope for DEHP on page 99/148						

Overall Quality Determination

High

Page 105 of 958

Study Citation: Duration: Exposure Route, Media, Path:	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality					
HERO ID:	1316201					
Domain	1010201	Metric	Rating	Comments		
Domain 1: Test Substand	stance					
	Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure. Measured concentrations deviated from reported nominal concentrations.		
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered		
		Administration		consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured at the initiation and termination of the experi-		
	Matria 10	Concentration Exposure Duration and Frequency	High	ment. Measured concentrations deviated from nominal concentrations.		
	Metric 10.	Exposure Duration and Frequency	nigii	toxicity test.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors and adequate to address the purpose of the study. For DEHP, a preliminary test was conducted, which indicated that DEHP was not toxic below its water solubility limit. A corroborative test was then conducted exposing the bluegill to a single replicated concentration of DEHP representing its limit of water solubility.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at the water solubility limit.		
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 blue gill in each test jar, and they were tested in duplicates.		

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

HERO ID: 1316201 Table: 2 of 2

continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Lepomis macrochirus</i>; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP) 1316201 				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.	
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.	
Domain 7: Data Presen	tation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	There was no mortality at the concentration tested. Therefore, statistical analysis was not conducted.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group. Negative findings were reported quantitatively (Table 4).	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	Non-definiti limit of wate	ve LC 50 values were reported based on a er solubility) conducted by exposing bluegil	corroborativ lls to a single	the test (following negative findings from a preliminary test at concentration below the replicated concentration of DEHP representing its limit of water solubility.	

Overall Quality Determination H

High

Study Citation:	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bulletin of Environmental					
Duration	Contamination and Toxicology 26(4):446-452.					
Fynosure Route	Overall Duration: 0 - 4 days (0-90n); Exposure Duration: 0 - 4 days (0-90n) Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)					
Media. Path:	Aquatic (freshwater); water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake foute)					
Taxa, Species, Age:	Vertebrate: Fish: Lenomis macrochirus: Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	18064					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Medium	Purity of the test substance was reported as greater than 80%.		
Domain 2: Test Design						
8	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. They also noted that "most of the LC50 values reported do not reflect [t]he actual concentrations of the chemical which were in solution in the diluent", because "the acute toxicity of most of the chemicals tested was at concentrations above their water solubility".		
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine.		
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured. They also noted that "most of the LC50 values reported do not reflect [t]he actual concentrations of the chemical which were in solution in the diluent", because "the acute toxicity of most of the chemicals tested was at concentrations above their water solubility".		
	Metric 10:	Exposure Duration and Frequency	High	Standard test durations were used (24h and 96h).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	No information is provided on the number of exposure groups and the spacing of expo- sure levels, but cited methods suggest using a minimum of 5 treatment levels.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The authors reported this as "precipitate". However, the highest reported LC50 is 2.1 mg/L, compared to the solubility reported in the DBP Final Scope of 11.2 mg/L at 25C.		
Domain 4: Test Organism						
·· · · · · · · · · · · · · · · ·	Metric 13:	Test Organism Characteristics	Medium	There are minor uncertainties about the source and characteristics of test organisms because the authors use a generalized description for all reported tests		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms for the 48 hours prior to testing.		
Continued on next page						

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Diethylhexyl Phthalate

HERO ID: 18064 Table: 1 of 1

b, R. J., Ells, S. J., Leblanc, G. A. (1981). Attion and Toxicology 26(4):446-452. ration: 0 - 4 days (0-96h); Exposure Duration reshwater); Water; Not determined by study at Fish; <i>Lepomis macrochirus</i> ; Not Applicable (xyl phthalate (DEHP) <u>Metric</u> Number of Organisms and Replicates per Group	cute toxicity of a: 0 - 4 days (0-9 uthors (i.e., chen (e.g., fungi or al (e.g., fungi or al Rating Low	priority pollutants to bluegill (Lepomis macrochirus). Bulletin of Environmental (6h) nical of interest in exposure water, but unable to determine exact uptake route) gae studies) or Not Reported Comments
ration: 0 - 4 days (0-96h); Exposure Duration eshwater); Water; Not determined by study at Fish; <i>Lepomis macrochirus</i> ; Not Applicable (xyl phthalate (DEHP) <u>Metric</u> Number of Organisms and Replicates per Group	a: 0 - 4 days (0-9 uthors (i.e., chen (e.g., fungi or al (e.g., fungi or al (e.g., fungi or al (e.g., fungi or al) (e.g., fungi or al)	6h) nical of interest in exposure water, but unable to determine exact uptake route) gae studies) or Not Reported Comments
eshwater); Water; Not determined by study at Fish; <i>Lepomis macrochirus</i> ; Not Applicable (xyl phthalate (DEHP) <u>Metric</u> Number of Organisms and Replicates per Group	uthors (i.e., chen (e.g., fungi or al Rating Low	nical of interest in exposure water, but unable to determine exact uptake route) gae studies) or Not Reported Comments
Fish; <i>Lepomis macrochirus</i> ; Not Applicable (xyl phthalate (DEHP) <u>Metric</u> Number of Organisms and Replicates per Group	(e.g., fungi or al Rating Low	gae studies) or Not Reported Comments Tan fick ware used, but no raplicates ware reported
Fish; <i>Lepomis macrochirus</i> ; Not Applicable (xyl phthalate (DEHP) <u>Metric</u> Number of Organisms and Replicates per Group	(e.g., fungi or al Rating Low	gae studies) or Not Reported Comments Ten fick were used, but no replicates were reported
xyl phthalate (DEHP) Metric Number of Organisms and Replicates per Group	Rating Low	Comments
xyl phthalate (DEHP) Metric Number of Organisms and Replicates per Group	Rating Low	Comments
Metric Number of Organisms and Replicates per Group	Rating Low	Comments
Metric Number of Organisms and Replicates per Group	Rating Low	Comments
Number of Organisms and Replicates per Group	Low	Ten fish were used but no replicates were reported
Replicates per Group		ten isn were used, but no replicates were reported.
Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
Outcome Assessment Methodology	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
		1
ontrol		
Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental condition
Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
alvsis		
Statistical Methods	High	Statistical methods were adequately described (method of moving average angles or Wilcoxon log probit).
Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group but results were described in the tables.
Explanation of Unexpected Outcomes	Medium	The occurrence of unexpected outcomes was not addressed.
	Outcomes Unrelated to Exposure alysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	Outcomes Unrelated to Exposure Medium alysis Statistical Methods High Reporting of Data Low Explanation of Unexpected Outcomes Medium

Study Citation:	Barrows, M	arrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish						
Duration	(Lepomis m	acrochirus). :379-392. ation: > 21 days: Exposure Duration: > 21	dave					
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	1							
Taxa, Species, Age:	Vertebrate; I	Fish; Lepomis macrochirus; Not Applicable	(e.g., fungi or alg	gae studies) or Not Reported				
Health Outcome:	ADME (bio	ADME (biotransformation)						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	18050							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name and molecular weight.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.				
Domain 2. Test Design								
Domain 21 Test Design	Metric 4:	Negative Controls	Low	Study authors reported using a concurrent negative control group, but acetone was used to dissolve the test chemical. Acetone was not reported as being used in the control.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported, although a background level of 20 cpm C-14 was detected prior to the exposure.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
	, . <u>.</u> .							
Domain 3: Exposure Ch	Matria 7:	Experimental System/Test Media	Uich	The experimental system and the methods for momentian of the test media wave de				
	Metric 7.	Preparation	nigii	scribed in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered				
		Administration	TT 1	consistently across study groups.				
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.				
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of the exposure was reported and appropriate for the study type (until a steady state was reached).				
	Metric 11:	Number of Exposure Groups/	N/A	Only one sublethal concentration was tested.				
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the water solubility limit.				
Domain 4: Test Organis	m							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source				
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.				
		Conditions	8	e				
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were thirty fish used with no replicates.				
Domain 5: Outcome As	sessment							

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Environmental Hazard Evaluation

HERO ID: 18050 Table: 1 of 1

		contin	nued from previo	us page			
Study Citation:	Barrows, M (Lepomis m	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (Lepomis macrochirus). :379-392.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biotransformation)						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	18050						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	Medium	It wasn't clear how steady state was determined. For example, if there was subsampling or a duplicate exposure.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Fish were fed during the exposure. This factor may influence the results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typically performed for this type of endpoint.			
	Metric 22:	Reporting of Data	Medium	Only the overall BCF was reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quali	ty Deterr	nination	Medium				

Study Citation: Duration: Exposure Route, Media Path:	Kirsch, P., N Overall Dura Aquatic (free	Kirsch, P., Munk, R. (1989). Report on the study of the acute toxicity. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	Fish; <i>Leuciscus idus L</i> .; golden variety; Not A	Applicable (e.g., fungi or a	lgae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	11328252	11328252					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice		т				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only as Palatinol AH, which is a synonym for DEHP.			
	Metric 2:	Test Substance Source	Low	The source of the DEHP was not explicitly stated, but the sponsor of the study was BASF Aktiengesellschaft. It was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity of the DEHP was reported to be 99.5%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which no test sub- stance was added to the dilution water.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in the table on page 7, and it was adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3: Exposure Ch	naracterization						
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Low	Little details were provided on the preparation of the test concentrations. It was reported the product was added to the test water without any pretreatment. A static procedure was reported to be used.			
	Metric 8:	Consistency of Exposure Administration	Low	Limited details on the preparation of the test substance were reported, this creates doubt on the consistency of the test. All exposures were reported to be static in similar test chambers with a photoperiod of 16L:8D. The temperature was maintained at 20C for the duration of the study.			
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if DEHP levels were analyzed in any of the test concentrations at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is typical of an acute toxicity study with fish.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups (5,000 and 10,000mg/L). This is lower than is typi- cal, and both exposure levels were above the water solubility limit.			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	The concentrations of both test levels were above the water solubility limit (5,000 and 10,000mg/L DEHP). It was not reported if a solvent was used. Study authors also reported undissolved oily test substance was visible at the water surface.			
Domain 4: Tast Orcania	m						
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the golden orfe was reported as Fischzucht Paul Eggers, D-2354 Hohen- westedt, FRG. The age of the folden orfe was not reported.			
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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 11328252 Table: 1 of 1

		00	ontinued from previous	page			
Study Citation: Duration: Exposure Route, Media. Path:	Kirsch, P., M Overall Dura Aquatic (fres	Kirsch, P., Munk, R. (1989). Report on the study of the acute toxicity. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	ish; Leuciscus idus L.; golden variety; Not A	Applicable (e.g., fungi or	algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	11328252						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The organisms were acclimated to the test vessels and test water for 3 days prior to the start of the study.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per treatment level and control. It appears as though there were no replicates, though this is not stated explicitly.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Water quality was monitored and reported for the duration of the test. The organisms were kept at 20C with a 16L:8D photoperiod. They were housed at a loading rate of 3.4g fish per liter of test water. Fish were not fed during the test; they were fed "growing feed" for the holding period prior to testing ad libitum.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-mortality in the form of LC50 values at various time points.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The fish were monitored for mortality at 1, 4, 24, 48, 72, and 96h.			
Domain 6: Confounding	/ Variable Cor	itrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Presenta	ation and Analy	ysis					
	Metric 21:	Statistical Methods	High	No mortalities occurred at any exposure level at any point in the study, though probit analysis was reported to be used for LC50 calculations.			
	Metric 22:	Reporting of Data	High	Raw data was reported in the table on page 7 of the PDF. Both exposure and control responses were reported here. LC50 calculations were reported on subsequent pages of the PDF.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This was a re received an u authors did n	Vietric 23: Explanation of Unexpected Outcomes High Study authors did not report any unexpected outcomes. Flis was a report on the acute toxicity of Palatinol AH (DEHP) to the golden orfe. LC50 values were calculated for 1, 4, 24, 48, 72, and 96h. This study received an unacceptable rating due to the test concentrations. Both test concentrations were well above the water solubility limit for DEHP, and the study withors did not report the use of a solvent. They did report that oily undiscolved test substance was visible at the water surface.					

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 5774391				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.	
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.	
	Metric 3:	Test Substance Purity	High	The DEHP was high-purity (>99%) from a commercial source.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No	
		0	U	solvents were used to necessitate a solvent control.	
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.	
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.	
	Metric 8:	Consistency of Exposure	High	Details of the exposure are provided and are consistent among study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.	
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for calculation of an LC50.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of the concen- trations was provided, and care was taken to ensure minimal degradation or loss of the test substance during experiments.	
Domain 4: Test Organisi	n				
-	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source, and test organism details were provided.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.	
Continued on next page					

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5774391 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Defoe, D. L. Environment	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:		•					
Taxa, Species, Age:	Vertebrate; F	Fish; Oncorhynchus mykiss; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest (LC50) was appropriate.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment					
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no variations or inconsistencies reported across study groups, and environ- mental conditions are provided.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	veie					
Domain 7. Data i leselit	Metric 21.	Statistical Methods	High	Statistical methods were appropriate			
	Metric 21:	Reporting of Data	Low	Results provided were minimal with authors noting specific concentrations and no acute toxicity			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The author provided discussion on different results measured in other papers.			
Additional Comments:	DEHP was n treatments. V	ot acutely toxic to trout at the highest tested Very limited data provided.	d concentratio	on (at/above water solubility). There were control, medium, and high test concentration			

Study Citation:	Cohle, P., St	tratton, J. (1992). Early Life-Stage Toxici	ty of DEHP (CA	S No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a		
Duration: Exposure Route, Media Path:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo Mortality Di-ethylhexyl phthalate (DEHP) 11328250					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not re- ported if the DEHP was analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7% The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The study authors reported the use of an appropriate negative control in which no DEHP was used.		
	Metric 5:	Negative Control Response	High	The negative control response of the preliminary study was reported in Table III and was adequate for the outcome of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into test chambers for the prelimi- nary study.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations. The test chambers were not described in detail, nor were the flow rates or other environmental conditions.		
	Metric 8:	Consistency of Exposure Administration	Low	Test chambers were not adequately described in the preliminary study, nor were other test conditions. Analytical measurements were performed on days 6 and 15 of the study. The nominal concentration of the highest concentration was reported to be 40ug/L, and the measured concentrations on days 6 and 15 were 1.1 and 0.89ug/L respectively. This large difference creates concern about the consistency of the exposure administration.		
	Metric 9:	Measurement of Test Substance Concentration	Low	Gas chromatography techniques were used for analytical measurements in the highest test concentration only. Other test concentrations were not measured in the preliminary study. The test concentrations measured were vastly different from the nominal concen- trations.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of the preliminary study was reported to be 18 days. This was adequate to determine definitive test concentrations.		
		Cont	inued on next pa	age		

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media Path:	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792 Flow-Through System. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rou				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 11328250	ish; <i>Oncorhynchus mykiss</i> ; Embryo I phthalate (DEHP)			
Domain		Metric	Rating	Comments	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the preliminary study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentration in the highest test level, which was the only level measurements were made. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.	
	Metric 12:	Testing at or Below Solubility Limit	Low	Test concentrations were believed to be below the water solubility level. Testing was done after the preliminary test, and the water solubility limit for the dilution water was determined to be 23ug/L, which would place the highest test concentration above the water solubility limit.	
Domain 4. Test Organisi	m				
Domain 1. Test Organisi	Metric 13:	Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the preliminary study.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were two replicates for each test concentration and control with 10 embryos each, for a total of 20 embryos for each test concentration.	
Domain 5: Outcome Ass	resement				
Domain 5. Outcome Ast	Metric 16:	Adequacy of Test Conditions	Medium	The characteristics of the soft blended water used as the dilution water were reported in Table I. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. Feeding regimen, temperature, DO, and other water quality parameters were not discussed in the preliminary study.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest–egg and fry mortality.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for the preliminary study were lim- ited. It was not reported when hatch was monitored or how often survival was checked for and determined.	
Domain 6: Confounding	/ Variable Cor	atrol			
Domain 0. Comounding	Metric 19:	Confounding Variables in Test	Low	It was not reported if the organisms were acclimated to test conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	

Domain 7: Data Presentation and Analysis

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HERO ID: 11328250 Table: 1 of 1

		continu	ed from previ	ious page		
Study Citation:	Cohle, P., S	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a				
	Flow-Through	gh System.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days			
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., cher	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Fish; Oncorhynchus mykiss; Embryo				
Health Outcome:	Mortality					
Chemical:	Di-ethvlhex	vl phthalate (DEHP)				
HERO ID:	11328250	11328250				
Domain		Metric	Rating	Comments		
	Metric 21:	Statistical Methods	Low	It was not reported if statistical analysis was conducted for the preliminary study. Raw data is provided in Table III.		
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response of the preliminary study are provided in Table III and are adequate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study authors did not provide measures of variability for this portion of the study.		
Additional Comments: This evaluation was for the preliminary early life-stage study on rainbow trout embryos. The preliminary study was conducted for 18 days and assessed percent hatch and survival of the fry. Little information was provided on when the organisms were monitored for hatch and survival. Nominal concentrations were drastically different from measured concentrations at the highest test level, which was the only test level monitored in the preliminary study.						
Overall Quality Determination Medium						

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1 Flow-Through System. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake				
Taxa, Species, Age:	Vertebrate; F	ish; Oncorhynchus mykiss; Embryo			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	I phthalate (DEHP)			
	11328230				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce		TT' 1		
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not re- ported if the DEHP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7% The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	Low	The study authors reported the use of a negative control. However, it was reported that control samples had low level DEHP contamination.	
	Metric 5:	Negative Control Response	High	The negative control response of the definitive study was reported in Table VIII and was adequate for the outcome of interest.	
	Metric 6:	Randomized Allocation	Low	It was reported the embryos were impartially allocated to the test chambers five at a time until all cups contained five eggs. This was continued until all chambers contained 50 eggs.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations, thus the low rating. Test chambers for embryos consisted of incubator cups suspended in the test chambers. Each replicate test chamber was 30.5cm x 15.7cm with a water depth of 15.7cm.	
	Metric 8:	Consistency of Exposure Administration	Low	Test concentrations were reported to have 25-52% recovery from nominal concentra- tions. The two lowest test levels were only sampled on days 0, 1, and 7 due to incon- sistencies in the measurements. The low and inconsistent recovery rates along with the detection of DEHP in the controls created concerns about consistency of the administra- tion.	
	Metric 9:	Measurement of Test Substance Concentration	Low	Gas-liquid chromatography techniques were used for analytical measurements. Samples were taken on days 0, 1, and 7, and then approximately every 7 days after that, as well as at test termination. Sampling was discontinued for the two lowest test levels beginning on day 14, at request of the study sponsor.	

Environmental Hazard Evaluation

HERO ID: 11328250 Table: 1 of 2

		conti	nued from previ	ous page				
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Cohle, P., St Flow-Throug Overall Dura Aquatic (fres Vertebrate; F	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a Flow-Through System. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo						
Health Outcome: Chemical: HERO ID:	Mortality Di-ethylhexyl phthalate (DEHP) 11328250							
Domain		Metric	Rating	Comments				
	Metric 10:	Exposure Duration and Frequency	Low	The study duration was 70 days total or until 35 days post hatch. This was shorter than is the typical 60 days post hatch. The study was ended early at the request of the study sponsor due to inconsistent analytical measurements.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the definitive study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentrations. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.				
	Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were reported to be below the water solubility limit of DEHP. Study authors tested solubility in the dilution water and determined it to be 23ug/L. The highest nominal concentration was reported to be 20ug/L, and the measured concentration was much lower than this.				
Domain 1. Test Organis	m							
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to exposure conditions prior to the start of the definitive study.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates for each test concentration and eight replicates for the con- trol. There were 50 embryos in each test chamber. After hatch, excess fry were dis- carded, and there were 25 fry per test chamber.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	The characteristics of the soft blended water used as the dilution water were reported in Table I. Water quality measurements were reported in Table VI. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. The fish were fed starting on day 47 of the study. They were fed brine shrimp nauplii for the first six days and then Salmon Starter was fed in addition to the brine shrimp.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-egg and fry mortality.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Total number of fry that hatched was determined on day 35 of the study. Fry mortality was monitored daily.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated to test conditions.				

Continued on next page ...

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Environmental Hazard Evaluation

		contin	ued from previ	ous page			
Study Citation:	Cohle, P., St	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a					
	Flow-Throug	Flow-Through System.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Oncorhynchus mykiss; Embryo					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	11328250						
Domain		Metric	Rating	Comments			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analysis" section and were appro- priate for the outcomes of interest.			
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response of the definitive study are pro- vided in Table VIII and are adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study authors did not provide measures of variability for this portion of the study.			
Additional Comments:	This evaluati and assessed consistency.	ion was for the definitive early life-stage stud percent hatch and survival of the fry. Nomin	ly on rainbow tr nal concentration	out embryos. The definitive study was conducted for 70 (35 days post hatch) days as were drastically different from measured concentrations creating concern about			
		••					

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route.	Cohle, P., Si Flow-Throug Overall Dura Aquatic (free	tratton, J. (1992). Early Life-Stage Toxic gh System. ation: > 21 days; Exposure Duration: > 2 shwater): Water: Not determined by study	ity of DEHP (CA 1 days authors (i.e., cher	S No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a nical of interest in exposure water, but unable to determine exact uptake route)
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Developmen Di-ethylhexy 11328250	Fish; <i>Oncorhynchus mykiss</i> ; Embryo tt/Growth yl phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not re- ported if the DEHP was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7%. The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.
Domain 2: Test Design				
-	Metric 4:	Negative Controls	Low	The study authors reported the use of a negative control. However, control samples had low level DEHP contamination.
	Metric 5:	Negative Control Response	High	The negative control response of the definitive study was reported in Table IX and was adequate for the outcome of interest. Control results were also reported in Figures 5 and 6.
	Metric 6:	Randomized Allocation	Low	It was reported the embryos were impartially allocated to the test chambers five at a time until all cups contained five eggs. This was continued until all chambers contained 50 eggs.
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations, thus the low rating. Test chambers for embryos consisted of incubator cups suspended in the test chambers. Each replicate test chamber was 30.5cm x 15.7cm with a water depth of 15.7cm.
	Metric 8:	Consistency of Exposure Administration	Low	Test concentrations were reported to have 25-52% recovery from nominal concentra- tions. The two lowest test levels were only sampled on days 0, 1, and 7 due to incon- sistencies in the measurements. The low and inconsistent recovery rates along with the detection of DEHP in the controls created concerns about consistency of the administra- tion.
	Metric 9:	Measurement of Test Substance Concentration	Low	Gas-liquid chromatography techniques were used for analytical measurements. Samples were taken on days 0, 1, and 7, and then approximately every 7 days after that, as well as at test termination. Sampling was discontinued for the two lowest test levels beginning on day 14, at request of the study sponsor.
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HERO ID: 11328250 Table: 2 of 2

		conti	nued from previ	ious page			
Study Citation: Duration: Exposure Route, Media Path:	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) Flow-Through System. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; H Developmer Di-ethylhex 11328250	Fish; <i>Oncorhynchus mykiss</i> ; Embryo ht/Growth yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
	Metric 10:	Exposure Duration and Frequency	Low	The study duration was 70 days total or until 35 days post hatch. This was shorter than is the typical 60 days post hatch. The study was ended early at the request of the study sponsor due to inconsistent analytical measurements.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the preliminary study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentrations. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.			
	Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were reported to be below the water solubility limit of DEHP. Study authors tested solubility in the dilution water and determined it to be 23ug/L. The highest nominal concentration was reported to be 20ug/L, and the measured concentration was much lower than this.			
Domain 1: Test Organi	sm						
Domain 4. Test organi	Metric 13:	Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the preliminary study.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates for each test concentration and eight replicates for the con- trol. There were 50 embryos in each test chamber. After hatch, excess fry were dis- carded, and there were 25 fry per test chamber.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	The characteristics of the soft blended water used as the dilution water were reported in Table I. Water quality measurements were reported in Table VI. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. The fish were fed starting on day 47 of the study. They were fed brine shrimp nauplii for the first six days and then Salmon Starter was fed in addition to the brine shrimp.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest–35d post hatch fry length and fry weight.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. At the termination of the study on day 35, fish were euthanized then blotted on paper towels to remove excess moisture. They were then weighed. Lengths were also determined at this point by measuring from the tip of the snout to the caudle peduncle.			
Domain 6: Confoundir	og / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated to test conditions.			

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Environmental Hazard Evaluation

HERO ID: 11328250 Table: 2 of 2

		contin	ued from previ	ous page		
Study Citation:	Cohle, P., St	tratton, J. (1992). Early Life-Stage Toxicity	of DEHP (CA	S No. 117-81-7) to Rainbow Trout (Oncorhynchus mykiss Walbaum 1792) in a		
	Flow-Throug	gh System.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	lays			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Fish; Oncorhynchus mykiss; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	11328250					
Domain	Metric		Rating	Comments		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analysis" section and were appropriate for the outcomes of interest.		
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response of the definitive study are pro- vided in Table IX and are adequate for the outcomes of interest. Data was also reported in Figures 5 and 6.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table IX.		
Additional Comments:	This evaluat and mean w consistency.	ion was for the definitive early life-stage stud et weight and length were assessed. Nomina	ly on rainbow tr ll concentration	out embryos. The definitive study was conducted for 70 (35 days post hatch) days s were drastically different from measured concentrations creating concern about		

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Study Citation: Duration: Exposure Route, Media, Path:	Defoe, D. L. Environment Overall Dura Aquatic (free	, Holcombe, G. W., Hammermeister, D. E tal Toxicology and Chemistry $9(5)$:623-63 ttion: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	., Biesinger, F 6. 1 days authors (i.e.,	X. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	Fish; Oncorhynchus mykiss; Embryo		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	5774391			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce		-	
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.
	Metric 3:	Test Substance Purity	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	High	Sufficient detail is provided regarding the experimental design as well as development
		Preparation	mgn	of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure	High	Details of exposure are provided and are consistent among study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for evaluation of hatchability, survival, and weight.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentra- tions was provided, and care was taken to ensure minimal degradation or loss of test substance during experiments.
Domain 4: Test Organisi	n			
	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source, and test organism details were provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.
		Cont	inued on nex	t page

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Diethylhexyl Phthalate

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Study Citation:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Oncorhynchus mykiss; Embryo					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest (survival) was appropriate.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment					
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no variations or inconsistencies reported across study groups and environ-			
	Matria 20.	Design and Procedures	Madium	Them is no information to suggest differences among groups			
	Metric 20.	Outcomes Onrelated to Exposure	Wiedlulli	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes, and variance was reported. Authors provided discussion on different results measured in other papers.			
Additional Comments:	Trout embry observed in t	os were exposed to DEHP for 90d with strout exposed to the mean DEHP concentra	ubsequent ev tion. Surviva	aluation of hatchability, survival, and larval weight. No differences in survival were al ranged from 93-100%.			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media. Path:	Defoe, D. L., Environment Overall Dura Aquatic (fres	Holcombe, G. W., Hammermeister, D. E. al Toxicology and Chemistry $9(5):623-63$ tion: > 21 days; Exposure Duration: > 2 hwater); Water; Not determined by study	., Biesinger, K 6. 1 days authors (i.e., 6	C. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	ish; Oncorhynchus mykiss; Embryo		
Health Outcome:	Development	/Growth		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	5774391			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.
	Metric 3:	Test Substance Purity	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure	High	Details of the exposure are provided and are consistent among study groups.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for evaluation of hatchability, survival, and weight.
		Spacing of Exposure Levels	0	
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of the concen- trations was provided, and care was taken to ensure minimal degradation or loss of test substance during experiments.
Domain 4. Test Organia	~			
Domain 4: Test Organisi	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source, and test organism details were provided.
	Metric 14:	Acclimatization and Pretreatment	Low	Minimal details on pretreatment were provided.
	Metric 15:	Conditions Number of Organisms and	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).
		Replicates per Group		

Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Defoe, D. L. Environment	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Oncorhynchus mykiss; Embryo						
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest was appropriate (hatchability, survival, weight).			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment					
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environ- mental conditions are provided.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes and, variance was reported. Author provided discussion on different results measured in other papers.			
Additional Comments:	Trout embry observed. Ha	os were exposed to DEHP for 90d with satchability ranged from 66-86%; Larval tro	subsequent e ut weight rai	valuations of hatchability and larval weight. No effects with DEHP exposure were nged from 599 mg in the high exposure tanks to 667 mg in the control tanks.			
Overall Qualit	ty Detern	nination	High				

Study Citation:	Bionomics,,	EG&G (1983). Acute toxicity of fourtee	en phthalate e	sters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report)
Destination	report no BV	V-83-3-1373.	0 4 1	(0.0(1))
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: $0 - 4$ days	(U-96n)
Exposure Route, Modio Dothy	Aquatic (free	snwater); water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Tava Species Age	Vertebrate: F	Fish: Oncorhynchus mykiss (Salmo gairdn	ari). A dult	
Health Outcome	Mortality	isii, Oncomynenus mykiss (Suino gurune	(<i>iii</i>), Adult	
Chemical:	Di-ethylbey	/l phthalate (DFHP)		
HERO ID:	5530771	(i philiadate (DEITI)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2:	Test Substance Source	High	The DEHP was obtained from General Electric Company, Hudson Falls, NY on 11 and 18 December 1981.
	Metric 3:	Test Substance Purity	High	"100% active ingredient," was reported. Though absolute purity is doubtful, this can be interpreted as an indication of very high purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were included.
	Metric 5:	Negative Control Response	High	There was no unacceptable mortality in the controls.
	Metric 6:	Randomized Allocation	Medium	Trout were randomly distributed among aquaria.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	A flow-through system with daily replenishment of solution was used. A detailed diluter design for mixing phthalates was described in Appendix I.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across substance groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Test concentrations were measured during experiment. Endpoints were based on mea- sured concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The 96-h acute exposure was an acceptable duration.
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations & the number of groups were acceptable to determine LC50
		Spacing of Exposure Levels		values.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The calculated LC50 (>0.32mg/L) was over the solubility limit for DEHP as reported in the Final Scope (0.28 mg/L).A dilution regimen for mixing the phthalates into solution was given in Appendix I. A combination of ultrasonication and mechanical mixing was used. Some of the high concentrations tested exhibited a visible film of undissolved phthalate. Although some measured concentrations were consistently lower than nominal, the measured concentrations were used to calculate LC50s.
Domain 1: Test Organia	m			
Domain 4: Test Organisi	Metric 13.	Test Organism Characteristics	High	Fish were obtained from commercial suppliers in Maryland & Montona. Lat numbers
	menie 13.		mgn	were given.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatization was for a minimum of 14 days in holding tanks.
		Cont	inued on nex	t page

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Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Bionomics,, report no BV	EG&G (1983). Acute toxicity of fourteer	n phthalate e	esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report)			
Duration:	Overall Dura	ation: 0 - 4 days (0-96h): Exposure Duration	on: 0 - 4 davs	(0-96h)			
Exposure Route.	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	1						
Taxa, Species, Age:	Vertebrate; F	Fish; Oncorhvnchus mvkiss (Salmo gairdne	ri); Adult				
Health Outcome:	Mortality	, , , , , , , , , , , , , , , , , , , ,					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5530771						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates per concentration.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The test conditions adequate for the husbandry of rainbow trout.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome was assessed appropriately (mortality).			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently among study groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences in conditions among study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to the exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	LC50 values were calculated by a customized computer program using moving average angle analysis, probit analysis, or binomial probability. Details of the program are not reported.			
	Metric 22:	Reporting of Data	High	Data was reported for all groups.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

ntinued from proviou

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Defoe, D. L. Environment Overall Dura Aquatic (fres Vertebrate; F Mortality Di-ethylhexy 5774391	, Holcombe, G. W., Hammermeister, D. E tal Toxicology and Chemistry 9(5):623-63 ation: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study Fish; <i>Oryzias latipes</i> ; Juvenile yl phthalate (DEHP)	., Biesinger, F 6. on: 0 - 4 days authors (i.e.,	K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. (0-96h) chemical of interest in exposure water, but unable to determine exact uptake route)
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.
	Metric 3:	Test Substance Purity	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No
				solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.
Domain 3: Exposure Ch	aracterization			
I II I	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure	High	Details of the exposure are provided and are consistent among study groups.
	Metric 9:	Measurement of Test Substance	High	The exposure concentrations were measured using appropriate methods.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for calculation of an LC50.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentra- tions was provided, and care was taken to ensure minimal degradation or loss of the test substance during experiments.
Domain 4: Test Organisi	n			
2	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source, and test organism details were provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.
		Cont	inued on nex	t page

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5774391 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Duration:							
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:		· · · · · · · · · · · · · · · · · · ·					
Taxa, Species, Age:	Vertebrate; Fish; Oryzias latipes; Juvenile						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest (LC50) was appropriate.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no variations or inconsistencies reported across study groups, and environ-			
		Design and Procedures		mental conditions are provided.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Author provided discussion on different results measured in other papers.			
Additional Comments:	DEHP was r test concentr	not acutely toxic to medaka at the highest action treatments. LC50 values were calculated	tested concer ated but mini	ntration (above water solubility). The authors assessed the control, medium, and high mal raw data was provided.			

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kim, E. J., I	Kim, J. W., Lee, S. K. (2002). Inhibition	of oocyte development in Japa	anese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate.
Dunation	Environment	t International 28(5):359-365.	10 dava	
Duration: Exposure Route	Aquatic (free	ation: 4 - 10 days; Exposure Duration: 4 -	vauthors (i.e., chemical of inter	est in exposure water, but unable to determine exact untake route)
Media. Path:	Aquatic (free	sinwater), water, not determined by study	authors (i.e., chemical of line	est in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	Fish: Oryzias latipes; Adult		
Health Outcome:	Mechanistic-	-Cell signaling/function		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	1303977			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce		TT 1	
	Metric 1:	Test Substance Identity	High	The rest chemical was identified by name.
	Metric 2:	Test Substance Source		The test substance identity was not analytically verified by the performing laboratory.
	Metric 5:	Test Substance Purity	High	The DEHP was reported as 99% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	There was no mention of control use in the methods, however, a control comparison was displayed in the results.
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were standard.
	Matria 12:	Spacing of Exposure Levels	Uigh	The average concentrations ware at an below the water colubility limit
	Wietric 12.	Testing at of Below Solubility Linit	rigii	The exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organisi	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Medium	The number of replicates was 10 fish per treatment.
		Replicates per Group		
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
			Continued on next page	

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

... continued from previous page

Study Citation:	Kim, E. J., H	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate.				
Duration	Environment	t International 28(5):359-365. ation: 4 - 10 days: Exposure Duration: 4 - 10	dave			
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study aut	hors (i.e., chemical of in	terest in exposure water, but unable to determine exact untake route)		
Media, Path:	riquite (ife		nors (n.e., encinear or m			
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Adult				
Health Outcome:	Mechanistic	Mechanistic-Cell signaling/function				
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1303977					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.		
Domain 6: Confoundin	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.		
Domain 7: Data Presen	tation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted - reports for the acute exposure/vitellogenin assay are mainly qualitative.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (free	(1999). Reproductive effects of phthalate est ation: 11 - 21 days; Exposure Duration: 4 - 1 shwater); Water; Not determined by study au	ers in Japanese medaka (0 days thors (i.e., chemical of ir	(Oryzias latipes). Doctoral Dissertation:137. nterest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 5489073	⁷ ish; <i>Oryzias latipes</i> ; Embryo yl phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	Nomenclature was reported in section 2.1. CAS number was not reported.
	Metric 2:	Test Substance Source	Low	The source was reported as from Sigma Aldrich, but it was not analytically verified by the lab.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The authors reported performing control and solvent controls.
	Metric 5:	Negative Control Response	Low	No control responses were reported for DEHP exposures.
	Metric 6:	Randomized Allocation	Low	The allocation was not random.
Domain 3: Exposure Ch	aracterization			
·	Metric 7:	Experimental System/Test Media Preparation	High	A static non-renewal 10 day exposure was conducted. At 10 days, replacement water was free of the compound. Authors used teflon rearing containers to reduce the leeching of test compound to the containers.
	Metric 8:	Consistency of Exposure	High	The exposures were the same across treatments and control groups.
	Metric 9:	Administration Measurement of Test Substance	Medium	The methods report that concentrations of DEHP were verified, but no concentrations are reported for DEHP exposures
	Metric 10:	Exposure Duration and Frequency	High	The exposure (10 days) was reported to cover the normal period of embryo development for this species. Modal hatch was recorded between 11-14 days.
	Metric 11:	Number of Exposure Groups/	Uninformative	The authors did not report any concentrations from the DEHP exposures.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The source was listed as from Carolina Biological Supply. These fish were bred to pro- duce embryos used in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Embryo media and test water conditions were reported on page 45/158.
	Metric 15:	Number of Organisms and Replicates per Group	Low	No animal numbers were reported for the acute toxicity bioassays, but the methods indicated they were peformed in triplicate.

Domain 5: Outcome Assessment

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5489073 Table: 1 of 2

		con	tinued from previou	s page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. Overall Dura Aquatic (free Vertebrate; F Mortality Di-ethylhexy 5489073	(1999). Reproductive effects of phthalate ester ation: 11 - 21 days; Exposure Duration: 4 - 10 shwater); Water; Not determined by study auth Fish; <i>Oryzias latipes</i> ; Embryo /l phthalate (DEHP)	s in Japanese medaka days ors (i.e., chemical of i	(Oryzias latipes). Doctoral Dissertation:137.
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	Embryo housing was detailed for the 10 day exposure, however, authors did not report water quality results to ensure adequate environmental conditions during embryogenesis.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for DEHP exposure was not reported.
Domain 6: Confoundin	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	No data were presented to indicate any outcomes from confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.
Domain 7: Data Presen	tation and Anal	ysis		
	Metric 21:	Statistical Methods	N/A	Authors were unable to perform statistics on these data. Negative findings were de- scribed in the text. "Acute Toxicity effects of up to 195.5 mg/L DEHP were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."
	Metric 22:	Reporting of Data	Low	There is not enough data on embryo mortality to relate to a dose-response gradient.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for mortality at specific concentrations to determine if there were unexpected outcomes.
Additional Comments:	The number examined in	of exposure groups and concentrations of DEI the Japanese medaka, and caused no observab	HP tested were not pr le lesions or death at a	ovided. "Acute Toxicity effects of up to 195.5 mg/L DEHP were also any of the tested concentrations."

Overall Quality Determination

Uninformative

Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route,	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Developmen Di-ethylhexy 5489073	ish; <i>Oryzias latipes</i> ; Embryo t/Growth l phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce		-		
	Metric 1:	Test Substance Identity	Low	Nomenclature was reported in section 2.1. CAS number was not reported.	
	Metric 2:	lest Substance Source	Low	The source was reported as from Sigma Aldrich, but it was not analytically verified by the lab.	
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	The authors reported performing control and solvent controls.	
	Metric 5:	Negative Control Response	Low	No control responses were reported for the DEHP exposures.	
	Metric 6:	Randomized Allocation	Low	The allocation method was not random.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	A static non-renewal 10 day exposure was conducted. At 10 days, replacement water was free of the compound. Authors used teflon rearing containers to reduce the leeching of test compound to the containers.	
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.	
	Metric 9:	Measurement of Test Substance Concentration	Medium	The methods report that concentrations of DEHP were verified, but no concentrations are reported for the DEHP exposures.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure (10 days) was reported to cover the normal period of embryo development for this species. Modal hatch was recorded between 11-14 days.	
	Metric 11:	Number of Exposure Groups/	Uninformative	The authors did not report any concentrations from the DEHP exposures.	
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organis	m				
C	Metric 13:	Test Organism Characteristics	High	The source was listed as from Carolina Biological Supply. These fish were bred to pro- duce embryos used in the study.	
	Metric 14:	Acclimatization and Pretreatment	High	Embryo media and test water conditions were reported on page 45/158.	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	No animal numbers were reported for the acute toxicity bioassays, but the methods indicated they were peformed in triplicate.	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Embryo housing was detailed for the 10 day exposure, however, authors did not report water quality results to ensure adequate environmental conditions during embryogenesis.	
			Continued on next page	•	

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5489073 Table: 2 of 2

		con	tinued from previou	is page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. Overall Dura Aquatic (frea Vertebrate; F Developmen Di-ethylhexy 5489073	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oryzias latipes</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 5480073				
Domain	5107075	Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	Methods for histology analysis were given in detail, but data on concentration specific incidence following exposure to DEHP were not provided.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for the DEHP exposure were not reported.		
Domain 6: Confoundin	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	No data were presented to indicate any outcomes from confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Authors were unable to perform statistics on these data. Negative findings were de- scribed in the text. "Acute Toxicity effects of up to 195.5 mg/L DEHP were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."		
	Metric 22:	Reporting of Data	Low	There is no data presented on incidence of lesions following DEHP exposure.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for lesions at specific concentrations to determine if there were unexpected outcomes.		

Additional Comments: The number of exposure groups and concentrations of DEHP tested were not provided. "Acute Toxicity effects of up to 195.5 mg/L DEHP were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."

Overall Quality Determination	Uninformative
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Study Citation: Duration: Exposure Route, Media, Path:	Chikae, M., estradiol on Overall Dura Aquatic (free	Ikeda, R., Hatano, Y., Hasan, Q., Morita the fry stage of medaka (Oryzias latipes). ation: > 21 days; Exposure Duration: 11 shwater); Water; Not determined by study	, Y., Tamiya, E. (2 Environmental To 21 days authors (i.e., chen	004). Effects of bis(2-ethylhexyl) phthalate, γ -hexachlorocyclohexane, and 17β - xicology and Pharmacology 18(1):9-12. nical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Oryzias latipes; Larvae						
Chamical:	Di ethylbey	vl phthalate (DEHD)						
HERO ID:	1333890	yr phulatate (DEIII)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		6					
	Metric 1:	Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.				
	Metric 2:	Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manu- facturer in Japan).				
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 1).				
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate (8%).				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	Authors provided limited details of the experimental systems and the test media prepara- tion. They described that DEHP was dissolved in ethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control so- lution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify the concentrations.				
	Metric 8:	Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors did not offer a rationale for the selected exposure groups. The number of exposure groups was adequate (0.01, 0.1, 1, and 10 ug/ml), but based on the mortality results (20% for 0.1, 1, and 10 ug/ml), the spacing of exposure levels might not have been most appropriate for a dose response assessment.				
		Con	tinued on next pa	ge				

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, γ -hexachlorocyclohexane, and 17 β estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 18(1):9-12. **Duration:** Overall Duration: > 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Larvae **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1333890 Rating Comments Domain Metric Metric 12: Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit (DEHP water solu-

			6	bility = 0.27 mg/L).
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 week for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors only reported using 20 fish but the use of replicates was not reported.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	The reporting of environmental conditions was insufficient to evaluate if they were ade- quate.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology to document mortality was not clearly described The authors reported that mortality was measured at six months, but it is not clear if this was cumulative mortality or mortality on one single day or throughout the sixth month period.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding	/ Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no evidence of differences among the study groups in environmental condi- tions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23.	Explanation of Unexpected Outcomes	Low	The study did not report any measure of variability for mortality

Diethylhexyl Phthalate

		continued from previous page	
Study Citation:	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q. estradiol on the fry stage of medaka (Oryzias	, Morita, Y., Tamiya, E. (2004). Effects latipes). Environmental Toxicology and	s of bis(2-ethylhexyl) phthalate, γ -hexachlorocyclohexane, and 17β - Pharmacology 18(1):9-12.
Duration:	Overall Duration: > 21 days; Exposure Durat	tion: 11 - 21 days	
Exposure Route,	Aquatic (freshwater); Water; Not determined	by study authors (i.e., chemical of intere	est in exposure water, but unable to determine exact uptake route)
Media, Path:			
Taxa, Species, Age:	Vertebrate; Fish; Oryzias latipes; Larvae		
Health Outcome:	Mortality		
Chemical:	Di-ethylhexyl phthalate (DEHP)		
HERO ID:	1333890		
Domain	Metric	Rating	Comments
Additional Comments:	The overall goal of this study was to examine	later-life health outcomes resulting from	n early-life exposure. The study examined the toxic effects of DEHP
	in adult Japanese medaka following a 3 weel	k exposure during the larval (fry) stage.	At their adult stage, mortality, sex ratio, and gonadosomatic index
	(GSI) were determined. This form evaluates t	he mortality data.	

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Chikae, M., estradiol on Overall Dura Aquatic (fres	Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y the fry stage of medaka (Oryzias latipes). En ation: > 21 days; Exposure Duration: 11 - 2 shwater); Water; Not determined by study at	<i>(</i> ., Tamiya, E. (2) nvironmental To 1 days uthors (i.e., chen	004). Effects of bis(2-ethylhexyl) phthalate, γ -hexachlorocyclohexane, and 17β - xicology and Pharmacology 18(1):9-12. nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Larvae		
Health Outcome:	Reproductive	e/Teratogenic		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	1333890			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.
	Metric 2:	Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manufacturer in Japan).
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.
Domain 2: Test Design			-	
	Metric 4:	Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 2).
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors provided limited details of the experimental systems and test media preparation. They described that DEHP was dissolved inethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control solution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were acceptable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organis	m			

Diethylhexyl Phthalate

Study Citation:

Exposure Route, Media, Path: Taxa, Species, Age:

Health Outcome:

Duration:

Chemical:

HERO ID:

...continued from previous page Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, γ-hexachlorocyclohexane, and 17β-estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 18(1):9-12. Overall Duration: > 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; Oryzias latipes; Larvae Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 1333890 Metric Rating Comments Metric 13: Test Organism Characteristics

Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 weeks for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors only reported using 20 fish, but the use of replicates was not reported.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was insufficient to evaluate if they were ade- quate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology to document growth (body weight) and develop- ment (sex ratio and GSI) was not described in detail. For example, the authors did not describe/report how GSI is calculated or if the weight was wet weight. However, these omissions unlikely affected the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding	y / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no evidence of differences among the study groups in environmental condi- tions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Present	ation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes and S.E. was reported.
Additional Comments:	The overall g in adult Japa (GSI) were o	goal of this study was to examine later-life h anese medaka following a 3 week exposure letermined. This form evaluates growth and	ealth outcomes during the larva development: b	resulting from early-life exposure. The study examined the toxic effects of DEHP al (fry) stage. At their adult stage, mortality, sex ratio, and gonadosomatic index ody weight, sex ratio, and GSI data. This form is for the sex ratio outcome.

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, γ-hexachlorocyclohexane, and 17β- estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 18(1):9-12. Overall Duration: > 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae Development/Growth Di-ethylhexyl phthalate (DEHP) 1333890			
Chemical: HERO ID:				
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.
	Metric 2:	Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manufacturer in Japan).
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.
Domain 2: Test Design			Ŧ	
	Metric 4:	Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 2).
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors provided limited details of the experimental systems and test media preparation. They described that DEHP was dissolved inethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control solution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were acceptable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
... continued from previous page

Diethylhexyl Phthalate

Study Citation:	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, γ-hexachlorocyclohexane, and 17β-									
Duration.	estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 18(1):9-12. Overall Duration: > 21 days: Exposure Duration: 11 - 21 days									
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)									
Media, Path:										
Taxa, Species, Age:	Vertebrate; F	ish; Oryzias latipes; Larvae								
Health Outcome:	Developmen	t/Growth								
Chemical:	Di-ethylhexy	l phthalate (DEHP)								
HERO ID:	1333890									
Domain		Metric	Rating	Comments						
	Metric 13:	Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.						
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 weeks for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.						
	Metric 15:	Number of Organisms and	Low	The authors only reported using 20 fish, but the use of replicates was not reported.						
		Replicates per Group								
Domain 5: Outcome Ass	essment									
	Metric 16:	Adequacy of Test Conditions	Low	The reporting of environmental conditions was insufficient to evaluate if they were ade- quate.						
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology to document growth (body weight) and develop- ment (sex ratio and GSI) was not described in detail. For example, the authors did not describe/report how GSI is calculated or if the weight was wet weight. However, these omissions unlikely affected the intended outcome.						
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.						
		Assessment								
Domain 6: Confounding	/ Variable Cor	ntrol								
	Metric 19:	Confounding Variables in Test	High	There was no evidence of differences among the study groups in environmental condi-						
	Matria 20	Design and Procedures	Mallin	tions.						
	Metric 20:	Outcomes Unrelated to Exposure	Wedium	attrition.						
Domain 7: Data Presents	tion and Anal	veic								
	Metric 21	Statistical Methods	High	Statistical methods were adequately described						
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group						
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes and S.E. was reported.						
Additional Comments:	The overall g in adult Japa	goal of this study was to examine later-life house medaka following a 3 week exposure	ealth outcomes a during the larva	resulting from early-life exposure. The study examined the toxic effects of DEHP l (fry) stage. At their adult stage, mortality, sex ratio, and gonadosomatic index						

(GSI) were determined. This form evaluates growth and development: body weight, sex ratio, and GSI data.

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Shioda, T., Wakabayashi, M. (2000). Evaluation of reproductivity of medaka (Oryzias latipes) exposed to chemicals using a 2-week reproduction test.						
	Water Scien	Water Science and Technology 42(7-8):53-60.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days				
Exposure Route, Modia Dath	Aquatic (free	shwater); Water; Not determined by study au	ithors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Taxa Species Age	Vertebrate [,] F	Fish: Orvzias latines: Adult					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1337871						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	Uninformative	The control hatch/unhatch rate presented in Figure 1e indicates that 50% or more of the control eggs did not hatch. This is very unacceptable for a comparison of treatment effects.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	Matria 7	Even minimantal System/Test Madia	Madium				
	Metric 7:	Preparation	Wiedrum	concentrations.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	Uninformative	The number of exposure groups and the spacing of exposure levels were not conducive			
		Spacing of Exposure Levels		to the purpose of the study.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolu- tion.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions			
		Conditions		were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome As	sessment						

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Shioda, T., Wakabayashi, M. (2000). Evaluation of reproductivity of medaka (Oryzias latipes) exposed to chemicals using a 2-week reproduction test. Water Science and Technology 42(7-8):53-60. **Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1337871 Domain Metric Rating Comments Metric 16: Adequacy of Test Conditions High Environmental conditions of the test system were conducive to the maintenance of organism health. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology reported the intended outcome of interest. Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure High There were no differences among groups that could influence the outcome assessment. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were adequately described. Metric 22: High Reporting of Data Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination

Uninformative

Study Citation: Duration:	Shioda, T., Wakabayashi, M. (2000). Effect of certain chemicals on the reproduction of medaka (Oryzias latipes). Chemosphere 40(3):239-243. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taya Species Age:	Vertebrate: Fish: Orvias latines: Adult					
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	683795	-				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The DEHP was sourced from GL Sciences.		
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	Medium	The biological responses were adequate, but they varied among replicates – as did those in the treatment groups.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations.		
	Metric 8:	Consistency of Exposure	Medium	Few details of the exposure administration were reported.		
	Metric 9:	Administration Measurement of Test Substance	Low	The exposure concentrations were not measured or the measurements were not reported.		
	Metric 10:	Exposure Duration and Frequency	Medium	Minor limitations in the exposure duration were identified. The exposure was not long enough to determine a dose response.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and the spacing of exposure levels were justified, but no dose response was reported.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4. Test Organis	m					
Domain 4. 10st Organis	Metric 13.	Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Conditions Number of Organisms and	Low	The numbers of test organisms and replicates were lower than the typical number used		
		Replicates per Group		in studies of the same type, almost unacceptable.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes.		
		Conti	nued on next pa	ge		

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Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route,	Shioda, T., V Overall Dura Aquatic (free	Shioda, T., Wakabayashi, M. (2000). Effect of certain chemicals on the reproduction of medaka (Oryzias latipes). Chemosphere 40(3):239-243. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa, Species, Age:	Vertebrate: H	rish: Orvzias latipes: Adult				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethvlhexy	vl phthalate (DEHP)				
HERO ID:	683795					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported.		
Domain 6: Confounding	g / Variable Co	ntrol				
·	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed, and the level of significance was 0.01.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quality Determination Medium						

Study Citation: Duration: Exposure Route,	Yang, W. K. growth and l Overall Dura Aquatic (free	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taya Species Age:	Vertebrate [,] F	Rish: Orwigs latings: Larvae				
Health Outcome:	Behavioral	isii, Oryzius iurpes, Earvae				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	4728529					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).		
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.		
	Metric 3:	Test Substance Purity	High	The study reported a test substance purity of >99.5%.		
Domain 2: Test Design						
8	Metric 4:	Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.		
	Metric 5:	Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 6.		
	Metric 6:	Randomized Allocation	Low	Randomization was indicated in the study report for the exposure groups, but the method was not indicated.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and test methods were described in adequate detail for this semi-static test (daily renewal). The larvae were moved to 24-well plates for the behavioral test, and the test procedure was adequately described.		
	Metric 8:	Consistency of Exposure	High	Exposures treatments were administered consistently across the control and test groups.		
	Metric 9:	Administration Measurement of Test Substance	High	Analytical concentrations were measured and reported in the Supplemental Material.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle control) were adequate to show results relevant to the outcome of interest.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organisi	m Metric 12.	Test Organism Characteristics	High	The test organism larvae were obtained from laboratory head tak		
	Metric 14	Acclimatization and Pretreatment	High	The test organism farvae were acclimated for 24-hours, and are treatment conditions were		
	mente 14.	Conditions	Ingii	the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of exposure test organisms is unclear, but there were at least 4 replicates of 4 test organisms per test concentration. There were 32 fish per test concentration for the locomotor activity behavior test.		

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 4728529 Table: 1 of 4

		contin	ued from p	previous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HFRO ID:	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae Behavioral Di-ethylhexyl phthalate (DEHP) 4728529				
Domain		Metric	Rating	Comments	
	Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	High High High	Organism housing and environmental conditions were adequate for the study. The outcome assessment methodology reported the intended outcomes of interest. The outcome assessment protocol was consistent across treatment groups.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.	
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.	
Domain 7: Data Present	ation and Anal Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Data for the locomotor activity were analyzed with nonparametric Kruskal-Wallis test. Data for all outcomes of interest and controls were presented. The authors adequately discussed unexpected outcomes in the paper.	
Additional Comments:	None				
Overall Qualit	ty Detern	nination	High		

Study Citation:	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval					
Duration: Exposure Route, Media, Path:	growth and I Overall Dura Aquatic (fres	Dverall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mechanistic- Di-ethylhexy 4728529	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology) Di-ethylhexyl phthalate (DEHP) 4728529				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).		
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.		
	Metric 3:	Test Substance Purity	High	The study reported a test substance purity of >99.5%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.		
	Metric 5:	Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 2-5.		
	Metric 6:	Randomized Allocation	Low	Randomization was indicated in the study report, but the method was not indicated.		
Damain 2. Enname Ch						
Domain 5: Exposure Ch	Matria 7	Environmental Surdaux/Teach Madia	II: -l-			
	Metric /:	Preparation	High	The experimental system and the test methods were described in adequate detail for this semi-static test (daily renewal).		
	Metric 8:	Consistency of Exposure	High	Exposures treatments were administered consistently across the control and test groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Mean-measured concentrations were provided in a supplemental Appendix A file, and based on test substance physical-chemical properties, measured concentrations are ex-		
				pected to be similar to nominal.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle		
	14 10	Spacing of Exposure Levels	TT' 1	control) were adequate to show results relevant to the outcome of interest.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.		
	Metric 14:	Acclimatization and Pretreatment	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were		
	Matria 15.	Conditions	Madium	the same for control and exposed organisms.		
	Metric 15:	Replicates per Group	Medium	organisms per test concentration; and 4 larvae per test concentration were pooled for the analysis.		
Domain 5: Outcome Ass	sessment					
Domain 5. Outcome As	Metric 16.	Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.		
		Conti	inued on nor	t nage		
Continued on next page						

Environmental Hazard Evaluation

HERO ID: 4728529 Table: 2 of 4

	continued from previous page					
Study Citation:	Yang, W. K. growth and l	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.				
Duration:	Överall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	- 21 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Larvae				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Oxidativ	e stress (inc	luding redox biology)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	4728529					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was consistent across treatment groups.		
Domain 6: Confounding	y / Variable Co	ntrol				
2 emain et comounant,	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.		
	Metric 22:	Reporting of Data	High	Data for all outcomes of interest and controls were presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.		
Additional Comments:	This evaluat	ion is specific to the mechanistic endpoints	examined ir	this experiment: gene expression (antioxidants and AChE biomarker).		
Overall Quality Determination High						

Study Citation:	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval			
Duration: Exposure Route, Media. Path:	growth and I Overall Dura Aquatic (fre	ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study	nways. Scien - 21 days authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Larvae		
Health Outcome: Chemical:	Developmen Di-ethylbey	nt/Growth vl.phthalate (DEHP)		
HERO ID:	4728529			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce		TT: 1	
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).
	Metric 2: Metric 3:	Test Substance Source	піgn Ціар	The test substance was obtained from Sigma-Aldrich, St Louis, MO. The study reported a test substance purity of $>00.5\%$
	Metric 5.	Test Substance Funty	nigii	The study reported a test substance purity of >99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.
	Metric 5:	Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 1 for growth and devel- opment.
	Metric 6:	Randomized Allocation	Low	Randomization was indicated in the study report, but the method was not indicated.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	High	The experimental system and test methods were described in adequate detail for this
	Wieure 7.	Preparation	mgn	semi-static test (daily renewal).
	Metric 8:	Consistency of Exposure	High	Exposures treatments were administered consistently across the control and test groups.
	Metric 9:	Administration Measurement of Test Substance	High	Measured analytical concentrations were provided in a supplemental Appendix and were slightly higher than nominal
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle control) were adequate to show results relevant to the outcome of interest
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	m			
2 onium 1. rost organis	Metric 13:	Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.
	Metric 14:	Acclimatization and Pretreatment	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms is unclear, but there were at least 4 replicates of 4 test organisms per test concentration.
Domain 5: Outcome Ar	acamant	I constructed to the		
Domain 5. Outcome Ass	Metric 16.	Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study
	Metric 17.	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest
	Metric 18:	Consistency of Outcome	High	The outcome assessment protocol was consistent across treatment groups.
Continued on next page				

Environmental Hazard Evaluation

HERO ID: 4728529 Table: 3 of 4

continued from previous page						
Study Citation:	Yang, W. K.	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval				
	growth and l	growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days			
Exposure Route, Modia Dath	Aquatic (free	shwater); water; Not determined by study a	uthors (1.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Tava Spacios Ago:	Vartabrata: F	Sish: Americas latinas: Larvas				
Hoalth Outcome:	Developmen	t/Growth				
Chemical.	Di-ethylbey	/ nhthalate (DEHP)				
HERO ID:	4728529	(i philadate (DEIII)				
Domain		Metric	Rating	Comments		
			0			
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences in environmental conditions or other factors among		
		Design and Procedures		the study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.		
	Metric 22:	Reporting of Data	High	Data for all outcomes of interest and controls were presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.		
Additional Comments:	None					
Overall Quality Determination High						

Study Citation:	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval				
Duration: Exposure Route, Media, Path:	growth and I Overall Dura Aquatic (fre	locomotion in medaka fish via multiple pat ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study	hways. Scien - 21 days authors (i.e.,	ce of the Total Environment 640-641:512-522. chemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Larvae			
Health Outcome:	Mortality				
HERO ID:	4728529	yi phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).	
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.	
	Metric 3:	Test Substance Purity	Hıgh	The study reported a test substance purity of >99.5%.	
Domain 2: Test Design					
· · ·	Metric 4:	Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.	
	Metric 5:	Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 1 for growth and devel- opment.	
	Metric 6:	Randomized Allocation	Low	Randomization was indicated in the study report, but the method was not indicated.	
Domain 2: Exposure Ch	aractorization				
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and test methods were described in adequate detail for this	
	Wietrie 7.	Preparation	mgn	semi-static test (daily renewal).	
	Metric 8:	Consistency of Exposure	High	Exposures treatments were administered consistently across the control and test groups.	
	Metric 0.	Administration Measurement of Test Substance	High	Massured applytical concentrations were provided in a supplemental Appendix and were	
	Wieure 9.	Concentration	Ingn	slightly higher than nominal.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure, 21 days with daily renewals, was appropriate for the study outcomes.	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle	
		Spacing of Exposure Levels		control) were adequate to show results relevant to the outcome of interest.	
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.	
	Metric 14:	Acclimatization and Pretreatment	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were the same for control and exposed organisms	
	Metric 15:	Number of Organisms and	Medium	The number of test organisms is unclear, but there were at least 4 replicates of 4 test	
		Replicates per Group		organisms per test concentration.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was consistent across treatment groups.	
Continued on next page					

Environmental Hazard Evaluation

HERO ID: 4728529 Table: 4 of 4

		contir	nued from p	previous page		
Study Citation:	Yang, W. K growth and	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days			
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	4728529					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.		
	Metric 22:	Reporting of Data	High	Data for all outcomes of interest and controls were presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.		
Additional Comments:	This form w	as for the mortality outcome reported in sec	ction 3.1.			
Overall Qualit	ty Deterr	nination	High			

Study Citation:	Chikae, M., Japanese me	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145.					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo Mortality					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1334110						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Low	The study provided few details on the exposure administration, notably, how long the embryos were exposed.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	Low	It was not clear if the post hatch solution contained DEHP.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a			
		Spacing of Exposure Levels		dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit with the use of ethanol.			
Domain 4: Test Organis	m						
U	Metric 13:	Test Organism Characteristics	Medium	There were minor reservations over using pet store fish to supply test embryos.			
	Metric 14:	Acclimatization and Pretreatment	Low	Eggs were pooled on different days for each treatment, which seems quite inconsistent for a pretreatment.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers were adequate although inconsistent; replicates were not reported.			
Domain 5: Outcome Ass	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.			
		Conti	nued on next pa	ge			

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Diethylhexyl Phthalate

continued from previous page					
Study Citation:	Chikae, M., l Japanese med	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145.			
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21 d	lays		
Exposure Route,	Aquatic (fres	hwater); Water; Not determined by study au	thors (i.e., chem	ical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vertebrate; F	ish; Oryzias latipes; orange-red variety; Eml	bryo		
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	1334110				
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		ited.	
Domain 6: Confounding	/ Variable Cor	ntrol			
c	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental	
		Design and Procedures		conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.	
Domain 7: Data Presentation and Analysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.	
Additional Comments:	Starting each	Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help.			

Overall Quality Determination

Medium

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Study Citation:	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145.					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; H Reproductiv Di-ethylhexy	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo Reproductive/Teratogenic				
HERO ID:	1334110	(- F)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		Ŧ			
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low Low	The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory, although it was obtained by Kanto Chemical.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design	N					
	Metric 4: Metric 5:	Negative Controls Negative Control Response	High	Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was reported and reasonable for assessed outcomes.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately re- ported but did not completely account for physical-chemical properties (e.g., period between renewals was greater than the half-life of a test substance that degrades in the system, however measured concentrations were provided for the treatment groups before the next renewal). The identified limitations are unlikely to have a substantial impact on results.		
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, but minor inconsistencies in ad- ministration of exposures among study groups were identified that are unlikely to have a substantial impact on results.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	Medium	It was not clear if the post hatch solution contained DEHP.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit with the use of ethanol.		
Domain 4: Test Organis	m					
-	Metric 13:	Test Organism Characteristics	Medium	There were minor reservations over using pet store fish to supply the test embryos.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Eggs were pooled on different days for each treatment, which seems quite inconsistent for a pretreatment.		
		Conti	nued on next pa	ge		

Diethylhexyl Phthalate

		contin	ued from previ	ous page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Chikae, M., Japanese me Overall Dura Aquatic (free Vertebrate; F Reproductive Di-ethylhexy 1334110	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 1334110				
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Numbers were adequate although inconsistent; replicates were not reported. "Spawning eggs were collected within 1–3 h after the lights were turned on. Under these conditions, approximately 40 eggs per day were pooled from ten pairs, and all eggs pooled in a day were assigned to one treatment."		
Domain 5: Outcome As	sassmant					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help. This form is for hatch success.					
Overall Quali	ty Detern	nination	Medium			

Study Citation:	Chikae, M.,	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of					
Duration: Exposure Route, Media Path	Japanese me Overall Dura Aquatic (free	werall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Oryzias latipes; orange-red variety; Embryo					
Health Outcome:	Developmen	t/Growth	-				
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1334110						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		-				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Low	The study provided few details on the exposure administration, notably, how long the embryos were exposed.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
		Concentration					
	Metric 10:	Exposure Duration and Frequency	Low	It was not clear if the post hatch solution contained DEHP.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit with the use of ethanol.			
Domain 4. Test Organis	m						
Domain 1. 10st Organis	Metric 13.	Test Organism Characteristics	Medium	There were minor reservations over using pet store fish to supply test embryos			
	Metric 14:	Acclimatization and Pretreatment	Low	Eggs were pooled on different days for each treatment, which seems quite inconsistent			
		Conditions		for a pretreatment.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers were adequate although inconsistent; replicates were not reported.			
Domain 5: Outcome As	sessment						
Bomain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade-			
	M 17		т	quate.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.			
Continued on next page							

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Diethylhexyl Phthalate

		contin	ued from previ	ous page		
Study Citation:	Chikae, M., Japanese me	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). Environmental Toxicology and Pharmacology 16(3):141-145.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; orange-red variety; Em	bryo			
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1334110	-				
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
		Assessment		ited.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	Additional Comments: Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help.					
		•				

Overall Quality Determination

Medium

Study Citation:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.					
Duration: Exposure Route, Media Path:	Aquatic (free	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa. Species. Age:	Vertebrate: F	Fish: Orvzias latines: Juvenile				
Health Outcome:	Mortality	, e your realized and the second s				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5774391					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.		
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.		
	Metric 3:	Test Substance Purity	High	The DEHP was high-purity (>99%) from a commercial source.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.		
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.		
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	High	Sufficient detail is provided regarding the experimental design as well as development		
		Preparation	mgn	of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.		
	Metric 8:	Consistency of Exposure	High	Details of the exposure are provided and are consistent among study groups.		
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.		
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for calculation of survival probability.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility though verification of the concen- trations was provided, and care was taken to ensure minimal degradation or loss of the test substance during experiments.		
Domain 4: Test Organist	n					
	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source, and test organism details were provided.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.		
	Continued on next page					

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Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Defoe, D. L. Environment	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	-						
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethvlhexy	/l phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest (survival) was appropriate.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment	-				
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environ- mental conditions are provided.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes and reported variance. Authors provided discussion on different results measured in other papers.			
Additional Comments:	Medaka were exposed to DEHP over 168 followed by assessment of survival across five sampling periods. Survival of fish exposed to DEHP was not reduced below control fish survival.						

Overall Quality Determination

High

Study Citation:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms.						
Duration	Environment	Environmental Toxicology and Chemistry 9(5):623-636.					
Duration: Exposure Route	Aquatic (free	tion: > 21 days; Exposure Duration: > 21 hwater): Water: Not determined by study :	uays authors (i.e., i	chemical of interest in exposure water, but unable to determine exact untake route)			
Media. Path:	Aquatic (fies	inwater), water, Not determined by study a	autions (i.e.,	enclinear of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vertebrate: F	ish: Orvzias latipes: Juvenile					
Health Outcome:	Development	t/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.			
	Metric 2:	Test Substance Source	Low	Test substance identity was not analytically verified.			
	Metric 3:	Test Substance Purity	High	DEHP was high-purity (>99%) from a commercial source.			
Domain 2: Test Design	N		TT' 1				
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.			
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with the control groups.			
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	Sufficient detail is provided regarding the experimental design as well as development			
		Preparation	e	of stock solutions. Water physiochemical characteristics were regularly measured and			
				reported. DEHP concentrations were measured and reported.			
	Metric 8:	Consistency of Exposure	High	Details of exposure are provided and are consistent among study groups.			
	Metric 9.	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods			
	mette).	Concentration	mgn	Exposure concentrations were inclusived using appropriate methods.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations and the duration of the study allowed allowed for evaluation of survival as well as weight changes.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility though verification of concentra- tions was provided, and care was taken to ensure minimal degradation or loss of test substance during experiments.			
Domain 4: Test Organisi	n Matria 12	Test Oreanism Chansetsistic	II:-L				
	Metric 13:	Test Organism Characteristics	High	lest organisms were obtained from a reliable source, and test organism details were provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	Minimal details on pretreatment were provided.			
	Metric 15:	Conditions Number of Organisms and	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
		Replicates per Group					

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 5774391 Table: 2 of 2

		conti	nued from p	revious page		
Study Citation:	Defoe, D. L. Environment	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Oryzias latipes; Juvenile				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5774391					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest was appropriate (survival, weight).		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.		
Domain 6: Confounding	g / Variable Coi	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environ- mental conditions are provided.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
Domain 7. Dua Present	Metric 21:	Statistical Methods	High	Statistical methods were appropriate		
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported		
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors provided discussion on different results measured in other papers.		
Additional Comments:	onal Comments: Medaka were exposed to DEHP over 168d followed by evaluation of survival and measurement of weights. Weights of DEHP-exposed medaka were lower than control fish weights.					
Overall Qualit	ty Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate.						
Duration:	Environment Overall Dura	t International 28(5):359-365. ation: > 21 days; Exposure Duration: > 1	21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by stud	y authors (i.e., chemical of inter	rest in exposure water, but unable to determine exact uptake route)			
Media, Path:	V	Siehe Ormaine Intin on Internetile					
Taxa, Species, Age: Health Outcome:	Vertebrate; F Mortality	rish; Oryzias latipes; Juvenile					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1303977						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice	— — — — — — — — — — — — — — — — — — —					
	Metric 1:	Test Substance Identity	High	The test chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The DEHP was reported as 99% pure.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	Low	There was no mention of control use in the methods; however, a control comparison was displayed in results.			
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were standard.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	Hıgh	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	sm						
C	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	30 fish per treatment were exposed, but only one tank (one technical replicate) was used.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported on to evaluate if they were adequate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.			
Continued on next page							

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Diethylhexyl Phthalate

HERO ID: 1303977 Table: 1 of 4

	continued from previous page					
Study Citation:	Kim, E. J., H	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate.				
	Environment	Environment International 28(5):359-365.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study auth	nors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1303977					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed		
		Assessment		consistently across study groups.		
Domain 6: Confoundin	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted. All data are provided for treatment and control fish in Table 1; however, there are no technical replicates.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	There was no measure of variability reported for the mortality endpoint (Table 1) be- cause all fish (30 at start) were housed in one tank so their was no technical replicate.		
Additional Comments: This evaluation is for mortality of fish from the chronic exposure (reported in Table 1).						

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.						
Duration: Exposure Route, Media, Path:	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Juvenile					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1303977						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The test chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The DEHP was reported as 99% pure.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	aractorization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Medium	The study provided limited details on the measures taken to appropriately prepare test			
		Preparation		concentrations.			
	Metric 8:	Consistency of Exposure	Low	The renewal schedule could not maintain a consistent test concentration.			
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured but didn't follow nominal values.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.			
	Matria 12	Spacing of Exposure Levels	Uich	Even over a concentrations, ware at an holow the water colubility limit			
	Metric 12:	Testing at of Below Solubility Linit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 30 fish per treatment, but only one tank (thus one technical replicate).			
Domain 5: Outcome Ass	sessment						
2 Smail 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions was not sufficiently reported on to evaluate if they were ade-			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			

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HERO ID: 1303977 Table: 2 of 4

		contin	ued from previo	us page			
Study Citation:	Kim, E. J., 1	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate.					
	Environmen	Environment International 28(5):359-365.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study at	uthors (i.e., chemi	cal of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Juvenile					
Health Outcome:	Developmen	nt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1303977						
Domain		Metric Rating Comments					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was not performed to test for differences in mortality.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was reported in Table 1.			
Additional Comments:	This evaluat	ion was for body weight.					
Overall Quality Determination Medium							

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration:	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International $28(5):359-365$. Overall Duration: > 21 days: Exposure Duration: > 21 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Juvenile					
Health Outcome:	Reproductive	el leratogenic					
HERO ID:	1303977	(i pitilalate (DEIII)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Test chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Hıgh	The DEHP was reported as 99% pure.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided limited details on the measures taken to appropriately prepare the test concentrations.			
	Metric 8:	Consistency of Exposure	Low	The renewal schedule could not maintain a consistent test concentration.			
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but didn't follow nominal values.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.			
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of replicates was not reported.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	The environmental conditions were not sufficiently reported on to evaluate if they were adequate.			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology addressed the intended outcome of interest. Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			

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HERO ID: 1303977 Table: 3 of 4

		contin	ued from previ	ous page			
Study Citation:	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Juvenile					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1303977						
Domain	Metric Rating Comments						
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed for GSI only.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group for GSI. Other results were in the narrative.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and a measure of variability for GSI was reported in Table 2			
Additional Comments:	Metric 23: This evaluation	Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and a m in Table 2			

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	 Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) 							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 1303977							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The test chemical was identified by name.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	The DEHP was reported as 99% pure.				
Domain 2. Test Design								
Domani 2. Test Design	Metric 4:	Negative Controls	Low	There was no mention of control use in the methods, however, a control comparison was displayed in results.				
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups was reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization		Ţ					
	Metric /:	Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were standard				
	Metrie 12	Spacing of Exposure Levels	IIIgn III-h					
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of replicates was not reported.				
Domain 5: Outcome Age	recoment							
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported on to evaluate if they were adequate.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.				
		С	ontinued on next page .					

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Juvenile **Health Outcome:** Mechanistic-Cell signaling/function Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1303977 Domain Metric Rating Comments Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Uninformative Statistical analysis was not conducted Metric 22: High Reporting of Data Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes Low The study did not report any measures of variability. Additional Comments: This evaluation is for serum proteins.

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Metcalfe C	D., Metcalfe, T. L., Kiparissis, Y., Koenig	. B. G., Khan	. C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of				
	chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicology							
	and Chemistry 20(2):297-308.							
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Larvae						
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	1333925							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	High	The chemical was identified as "the phthalic acid, diethylhexyl phthalate (DEHP)."				
	Metric 2:	Test Substance Source	High	The chemical was purchased from Sigma-Aldrich.				
	Metric 3:	Test Substance Purity	Low	Purity was not reported.				
Domain 2: Test Design								
U	Metric 4:	Negative Controls	High	A solvent (acetone) control was included in the study.				
	Metric 5:	Negative Control Response	High	Control responses were shown in Table 5.				
	Metric 6:	Randomized Allocation	Low	Authors did not state random allocation.				
Domain 2. Expeditor Ch	anastanization							
Domain 5. Exposure Ci	Matria 7.	Europimontal System/Test Madia	High	Environmental and Million and a second la described in the aview of second in				
	Metric 7.	Dreparation	nigii	cited methodology (Gray and Metcalfe 1907)				
	Metric 8	Consistency of Exposure	High	Datails of avposure administration were adequately explained and appeared to be consis				
	Mettre 6.	A dministration	Ingn	tent				
	Metric 9.	Measurement of Test Substance	Low	The DEHP concentration was not reported (authors attempted to measure but were un-				
	Methe 9.	Concentration	Low	successful due to background contamination)				
	Metric 10:	Exposure Duration and Frequency	High	This was approximately a 90 day exposure (until fish reached 1.5 cm in length) with renewal of dosing solution every two days				
	Metric 11.	Number of Exposure Groups/	Low	Different DEHP doses are stated in Table 5 $(0.5, 1)$ and 5 ug/L) vs DEHP doses stated in				
		Spacing of Exposure Levels	Low	Tables 1 and 6 (500, 1000, 5000 ug/L). It is unclear if doses stated in Table 5 are in the wrong units				
	Metric 12:	Testing at or Below Solubility Limit	Low	It is unclear if the doses reported in Table 5 are in the correct units. This would affect whether DEHP exceeded solubility in water				
				whether DEFIT exceeded solubility in water.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	The source of the stock were described in the cited paper (Gray and Metcalfe 1997). Au- thors did not report separating males from females or equalizing the number of starting males and females at initiation of experiment.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Authors report rearing eggs in ERM in petri dishes to hatch and then starting the exposure in a static renewal system 1 day post-hatch. Acclimation to the static renewal system was not stated.				

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Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Metcalfe, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 20(2):297-308. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; H	Fish; Oryzias latipes; Larvae					
Health Outcome: Chemical: HERO ID:	Developmen Di-ethylhexy 1333925	t/Growth /l phthalate (DEHP)					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 60 fish per treatment with no tank replication.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Housing conditions were adequately stated in the primary paper and in the cited reference (Gray and Metcalfe 1997).			
	Metric 17:	Outcome Assessment Methodology	Low	The methodology was not described for measurement of total length, wet weight, or calculation of condition factor.			
	Metric 18:	Consistency of Outcome Assessment	High	Measurements were taken when fish reached 1.5 cm in length.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Authors state significant difference (or lack thereof) in Table 5, but there was no expla- nation of what statistical test was conducted.			
	Metric 22:	Reporting of Data	High	Total length and wet weight are shown in Table 5 for DEHP treatments and controls.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The averages and associated standard deviation shown in Table 5 seem reasonable for DEHP treatments and control treatment.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Low				

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HERO ID: 1333925 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media, Path:	Metcalfe, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicolog, and Chemistry 20(2):297-308. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate: Fish; <i>Oryzias latipes</i> : Larvae						
Taxa, Species, Age:							
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1333925						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified as "the phthalic acid, diethylhexyl phthalate (DEHP)."			
	Metric 2:	Test Substance Source	High	The chemical was purchased from Sigma-Aldrich.			
	Metric 3:	Test Substance Purity	Low	The purity was not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	A solvent (acetone) control was included in the study.			
	Metric 5:	Negative Control Response	High	Control responses were shown in Table 6.			
	Metric 6:	Randomized Allocation	Low	Authors did not state random allocation.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	Environmental conditions were adequately described in the primary reference and cited methodology (Gray and Metcalfe 1997).			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were adequately explained and appeared to be consis- tent.			
	Metric 9:	Measurement of Test Substance Concentration	Low	The DEHP concentration was not reported (authors attempted to measure but were un- successful due to background contamination).			
	Metric 10:	Exposure Duration and Frequency	High	This was approximately a 90 day exposure (until fish reached 1.5 cm in length) with renewal of dosing solution every two days.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three doses, spanning an order of magnitude (0.5-5 mg/L), is a limited concentration range for observing effects.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The reported DEHP solubility in water is approximately 0.3 mg/L, authors tested 0.5-5 mg/L in water (the acetone solvent may have increased DEHP solubility in water above 0.3 mg/L but it is unclear if 5 mg/L solubility was achieved).			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	The source of the stock was described in a cited paper (Gray and Metcalfe 1997). Au- thors did not report separating males from females or equalizing the number of starting males and females at initiation of the experiment.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Authors report rearing eggs in ERM in petri dishes to hatch and then starting the ex- posure in a static renewal system 1 day post-hatch. Acclimation to the static renewal system was not stated.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 60 fish per treatment with no tank replication.			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Metcalfe, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryzias latipes). Environmental Toxicology and Chemistry 20(2):297-308.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Larvae					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1333925						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Housing conditions were adequately stated in the primary paper and in a cited reference (Gray and Metcalfe 1997).			
	Metric 17:	Outcome Assessment Methodology	High	Methodology for determining the presence of ova-testis was described.			
	Metric 18:	Consistency of Outcome Assessment	High	Measurements were taken when fish reached 1.5 cm in length.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Low	The numbers of fish in 1 mg/L and 0.5 mg/L DEHP treatments were almost half of those in the control and 5 mg/L DEHP treatments.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Authors stated significant difference (or lack thereof) in Table 6, but there was no expla- nation of what statistical test was conducted.			
	Metric 22:	Reporting of Data	High	The number of females and males (and number of males with TO) are shown in Table 6 for DEHP treatments and control.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	Chen, X., Xu phthalates ar Overall Dura Aquatic (free	Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mechanistic- Di-ethylhexy 2298079	Vertebrate; Fish; <i>Oryzias melastigma</i> ; ChgH-EGFP; Larvae Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 2298079						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The CAS numbers and structures for BBP, DBP, DEHP, DIDP, and DINP were reported.				
	Metric 2:	Test Substance Source	High	The sources were reported.				
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Both a blank control and a solvent control were used in the acute bioassays.				
	Metric 5:	Negative Control Response	High	Control responses (blank and solvent) are shown in Figure 3. Positive control responses are shown in Figures 3, 4, and 5.				
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.				
Domain 3: Exposure Ch	aracterization		-					
	Metric 7:	Experimental System/Test Media Preparation	Low	Preparation of the test substances and dilution into the test medium were not well de- scribed.				
	Metric 8:	Consistency of Exposure	High	Exposures appear to have been administered consistently.				
	Metric 9:	Administration Measurement of Test Substance	Low	Concentrations are reported as nominal.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures were 24-hr for embryos, shorter than typical 72-96 hr utilized in other trans- genic fish embryo studies.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five concentrations were utilized in the pre-testing, from which one concentration per phthalate was utilized in the formal testing.				
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations utilized in the formal testing did not exceed solubility (BBP and DPB) and slightly exceeded solubility (DIDP, DINP, DEHP). Methanol was utilized to increase solubility.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	The source for the transgenic medaka was cited to Chen et al 2007 and Cheng and Chen 2013, but it was not well described in the cited sources.				
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of embryos in 24-well plates was not reported.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each concentration was tested in triplicate with eight embryos per replicate.				

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		contin	ued from previo	us page			
Study Citation:	Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.						
Duration:	Overall Dur	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias melastigma; ChgH-EGFP; Lar	vae				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Receptor	binding/ regulati	on of receptor activity-Endocrine toxicity-Reproductive/Teratogenic			
Chemical:	Di-ethylhex	yl phthalate (DEHP)					
HERO ID:	2298079						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	The 24 well plates were described, but no other details on environmental conditions were provided.			
	Metric 17:	Outcome Assessment Methodology	Medium	Measurement of GFP in the liver of embryos was described in the methods. Anesthesia of embryos prior to imaging was not described.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and control groups at 72 hr of exposure.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate that animal health or attrition interfered with the bioas- say.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	The authors utilized Student's t-tests (alpha = 0.05) to determine significant difference of phthalate/E2 cotreatment activity from E2 activity. Other data analysis was performed according to ISO 20281.			
	Metric 22:	Reporting of Data	Medium	Solvent control and positive control data were shown in Figure 3. Responses for the BBP treatment were shown in Figure 4, and co-treatment responses for DBP, DEHP, DIDP, and DINP were shown in Figure 5.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Only one treatment concentration was reported (1.5 mg/L), but it was reported with mean +/- SEM.			
Additional Comments:	This form a	pplies to BBP, DBP, DEHP, DIDP, and DINF					
Overall Qualit	ty Deterr	nination	Medium				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84.							
	Overan Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
laxa, Species, Age:	Vertebrate; F	ish; Pelleobagrus fulviaraco; Adult						
Health Outcome:	Development	/Growth						
Chemical:	Di-ethylhexy	I phthalate (DEHP)						
HERO ID:	4742097							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No CASRN, structure, or other chemical descriptors were reported.				
	Metric 2:	Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they mea- sured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.				
	Metric 3:	Test Substance Purity	Low	The test substance purity/grade was not reported in the study.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.				
	Metric 5:	Negative Control Response	High	The researchers reported the negative control response adequately.				
	Metric 6:	Randomized Allocation	Low	The study authors did not report randomized allocation of the study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.				
	Metric 8:	Consistency of Exposure	High	The test substance was administered consistently across the study groups.				
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured using HPLC on a weekly basis.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration of 56 days was appropriate for the study type and assessment of growth performance.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control.				
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.				

Domain 4: Test Organism

				ierous page	
Study Citation:Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Phy Toxicology and Pharmacology 202:79-84.Duration:Overall Duration: > 21 days; Exposure Duration: > 21 daysExposure Route, Media, Path:Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Taxa, Species, Age:	Vertebrate; F	Fish; Pelteobagrus fulvidraco; Adult			
Health Outcome:	Developmen Di athylhavi	t/Growth			
HERO ID.	4742097	(DEHF)			
Domain	1112091	Metric	Pating	Comments	
Domani	Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details not provided, the fish for the study were selected based on uniformity of weight.	
	Metric 14:	Acclimatization and Pretreatment	High	The fish were uniformly acclimated to test conditions.	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to char- acterize toxicological effects.	
Domain 5. Outcome A	agament				
Domain 5: Outcome A	Metric 16:	Adequacy of Test Conditions	Medium	The size of the test tank was not specified, so it is unclear whether it was adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.	
	Metric 17:	Outcome Assessment Methodology	High	The researchers looked at final body weight, weight gain, and specific growth rate of the test organisms to observe exposure effects on growth and development.	
	Metric 18:	Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for growth and de- velopment.	
Domain 6: Confoundin	g / Variable Coi Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups that could affect outcome assessment, except that the authors did not report control impurities (ambient levels of DEHP) or the source of water used in the exposures which could potentially affect some of the results.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presen	tation and Anal	ysis Statistical Mathada	Uiah	The study moults ware measured as means with standard among and an in-the standard	
	Metric 21:	Statistical Methods	High	way ANOVA and Duncan's Multiple Range test as merited.	
	Metric 22:	Reporting of Data	High	The data for all outcomes were presented (section 3.1, Table 2).	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in the study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these	

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4742097 Table: 1 of 4

Study Citation: Yua to A	nn, L., Li, M., Meng, F., Gong, Y., Qian	V Shi C Wang B (2017) C	
Tox	Aeromonas hydrophila of juvenile yellow icology and Pharmacology 202:79-84.	catfish exposed to di-2-ethylhexyl	browth, blood health, antioxidant status, immune response and resistance l phthalate (DEHP). Comparative Biochemistry and Physiology - Part C:
Duration: Ove	erall Duration: > 21 days; Exposure Dura	tion: > 21 days	
Exposure Route, Aqu	uatic (freshwater); Water; Not determined	by study authors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)
Media, Path:			
Taxa, Species, Age: Vert	tebrate; Fish; Pelteobagrus fulvidraco; Ac	lult	
Health Outcome: Dev	elopment/Growth		
Chemical: Di-e	ethylhexyl phthalate (DEHP)		
HERO ID: 474	2097		
Domain	Metric	Rating	Comments
Additional Comments: This resp perf	s study investigated the effects of various ponses, and resistance to the Aeromonas formance assessment (final body weight, v	levels of DEHP exposure on grow hydrophila challenge on yellow ca weight gain, specific growth rate, fe	th performance, blood parameters, antioxidant enzyme activities, immune tfish Pelteobagrus fulvidraco. This form was used to evaluate the growth eed efficiency ratio, and hepatosomatic index; Section 2.1 and Table 2).

Overall Quality Determination

High

Study Citation:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C:
	Toxicology and Pharmacology 202:79-84.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route,	Aquatic (freshwater); Water; Injection, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake
Media, Path:	route)
Taxa, Species, Age:	Vertebrate; Fish; Pelteobagrus fulvidraco; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	4742097

Domain		Metric	Rating	Comments
Domain 1: Test Substance	e			
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No CASRN, structure, or other chemical descriptors were reported.
	Metric 2:	Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they mea- sured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.
	Metric 3:	Test Substance Purity	Low	The test substance purity/grade was not reported in the study.
Domain 2: Test Design				
U	Metric 4:	Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.
	Metric 5:	Negative Control Response	High	The researchers reported the negative control response adequately.
	Metric 6:	Randomized Allocation	Low	While the study authors reported randomized allocation of the bacterial challenge, they did not report randomized allocation of the DEHP exposure.
Domain 3: Exposure Cha	racterization			
1	Metric 7:	Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.
	Metric 8:	Consistency of Exposure	High	The test substance was administered consistently across the study groups.
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using HPLC on a weekly basis.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration of 56 days was adequate for the study type and to determine DEHP impacts on blood parameters, antioxidant enzyme activities, immune responses, and resistance to the Aeromonas hydrophila challenge.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.

Domain 4: Test Organism

Diethylhexyl Phthalate

		contin	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	ly Citation: Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resista to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part Toxicology and Pharmacology 202:79-84. ation: Overall Duration: > 21 days; Exposure Duration: > 21 days osure Route, Aquatic (freshwater); Water; Injection, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact upt lia, Path: route) a, Species, Age: Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult Ith Outcome: Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function mical: Di-ethylhexyl phthalate (DEHP) RO ID: 4742097						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details were not provided, the fish for the study were selected based on uniformity of weight.			
	Metric 14:	Acclimatization and Pretreatment	High	The fish were uniformly acclimated to test conditions.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to char- acterize toxicological effects.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The size of test tanks was not specified, so it is unclear whether they were adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The researchers reported effects on test organism hematological parameters and antiox- idant enzyme activity; they also tested the immune response of control and test groups when challenged with bacterial infection. The assessment methodologies (catalase ac- tivity, total antioxidant capacity, superoxide dismutase activity, glutathione peroxidase activity, serum complement C3 and C4, total immunoglobulin contents, respiratory burst, mRNA levels of TLR5 and MYD88) were reported and adequate for the intended outcome.			
	Metric 18:	Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for total serum pro- tein and other hematological parameters, enzyme activity, and immune response.			
Domain 6: Confounding	/ Variable Con	trol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups that could affect outcome assessment, except that the authors did not report control impurities (ambient levels of DEHP) or the source of water used in the exposures.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain 7: Data Presenta	ation and Analy	/\$15					
	Metric 21:	Statistical Methods	High	The study results were presented as means with standard errors and analyzed with one- way ANOVA and Duncan's Multiple Range test as merited.			
	Metric 22:	Reporting of Data	High	The data for all outcomes were presented (Tables 3, 4, 5 and Figures 1 and 3).			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these were adequately discussed by the study authors.			

Diethylhexyl Phthalate

HERO ID: 4742097 Table: 2 of 4

	continued from previous page					
Study Citation:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Injection, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake					
Media, Path:	route)					
Taxa, Species, Age:	Vertebrate; Fish; Pelteobagrus fulvidraco; Adult					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	4742097					
Domain	Metric Rating Comments					
Additional Comments:	This study investigated the effects of various levels of DEHP exposure on growth performance, blood parameters, antioxidant enzyme activities, immune responses, and resistance to the Aeromonas hydrophila challenge on yellow catfish Pelteobagrus fulvidraco. This form was used to evaluate the assessment on blood parameters, antioxidant enzyme activities, immune responses and resistance to the Aeromonas hydrophila challenge.					

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media Path:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 4742097	Fish; <i>Pelteobagrus fulvidraco</i> ; Adult yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	TThe test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No CASRN, structure, or other chemical descriptors were reported.		
	Metric 2:	Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they mea- sured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.		
	Metric 3:	Test Substance Purity	Low	The test substance purity/grade was not reported in the study.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.		
	Metric 5:	Negative Control Response	High	The researchers reported the negative control response adequately.		
	Metric 6:	Randomized Allocation	LOW	The study authors did not report randomized allocation of the study groups.		
Domain 3. Exposure Ch	aracterization					
Domain 5. Exposure et	Metric 7:	Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.		
	Metric 8:	Consistency of Exposure	High	The test substance was administered consistently across the study groups.		
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using HPLC on a weekly basis.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration of 56 days was adequate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control. Most importantly for the mortality outcome, the researchers did not test a range of concentrations to determine mortality.		
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.		
Domain 1. Test Organia	m					
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details were not provided, the fish for the study were selected based on uniformity of weight.		
		C	Continued on next page .	··		

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4742097 Table: 3 of 4

Study Citation:							
Duration:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84. Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; F	ish; Pelteobagrus fulvidraco; Adult					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	4742097						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	High	The fish were uniformly acclimated to test conditions.			
	Metric 15:	Conditions Number of Organisms and Penlicates per Group	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to char- acterize toxicological effects			
		Replicates per Gloup					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The size of the test tank was not specified, so it is unclear whether it was adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.			
	Metric 17:	Outcome Assessment Methodology	Uninformative	The researchers did not describe the mortality methodology. They did not describe a range-finding test. There was no rationale offered for the mortality assessment because examining mortality due to DEHP exposure was not a goal of the study.			
	Metric 18:	Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for mortality.			
Domain 6: Confounding	/ Variable Cor	atrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups that could affect outcome assessment, except that the authors did not report control impurities (ambient levels of DEHP)			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain /: Data Presenta	Metric 21	ysis Statistical Methods	Hioh	The study results were presented as means with standard errors and analyzed with one-			
	Weute 21.	Statistical Methods	mgn	way ANOVA and Duncan's Multiple Range test as merited.			
	Metric 22:	Reporting of Data	High	The data for all outcomes were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these were adequately discussed by the study authors.			

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4742097 Table: 3 of 4

		continued from previous page	
Study Citation:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian,	Y., Shi, G., Wang, R. (2017). Growth, blood heal	th, antioxidant status, immune response and resistance
	to Aeromonas hydrophila of juvenile yellow	catfish exposed to di-2-ethylhexyl phthalate (DEHF	P). Comparative Biochemistry and Physiology - Part C:
	Toxicology and Pharmacology 202: /9-84.		
Duration:	Overall Duration: > 21 days; Exposure Durati	on: > 21 days	
Exposure Route,	Aquatic (freshwater); Water; Not determined b	by study authors (i.e., chemical of interest in exposure	re water, but unable to determine exact uptake route)
Media, Path:			
Taxa, Species, Age:	Vertebrate; Fish; Pelteobagrus fulvidraco; Adu	ılt	
Health Outcome:	Mortality		
Chemical:	Di-ethylhexyl phthalate (DEHP)		
HERO ID:	4742097		
Domain	Metric	Rating	Comments
Overall Quali	ty Determination	Uninformative	

Study Citation: Duration: Exposure Route, Madia Path:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; H Behavioral Di-ethylhex 4742097	Fish; <i>Pelteobagrus fulvidraco</i> ; Adult yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	The test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No		
	Metric 2:	Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they mea- sured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.		
	Metric 3:	Test Substance Purity	Low	The test substance purity/grade was not reported in the study.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.		
	Metric 5:	Negative Control Response	High	The researchers reported the negative control response adequately.		
	Metric 6:	Randomized Allocation	Low	The study authors did not report randomized allocation of the study groups.		
Domain 3: Exposure Ch	aracterization					
·	Metric 7:	Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.		
	Metric 8:	Consistency of Exposure	High	The test substance was administered consistently across the study groups.		
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using HPLC on a weekly basis.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration of 56 days was adequate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control. Most importantly for the mortality outcome, the researchers did not test a range of concentrations to determine mortality.		
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.		
Domain 4. Test Organis	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details were not provided, the fish for the study were selected based on uniformity of weight.		
		(Continued on next page .			

Diethylhexyl Phthalate

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Study Citation: Duration:	Yuan, L., Li to Aeromona Toxicology a Overall Dura	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to Aeromonas hydrophila of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84. Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fres	shwater); Water; Not determined by study aut	hors (i.e., chemical of in	nterest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Pelteobagrus fulvidraco; Adult					
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	4742097						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	High	The fish were uniformly acclimated to test conditions.			
	Metric 15	Conditions Number of Organisms and	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to char-			
	Methe 15.	Replicates per Group	Wiedium	acterize toxicological effects.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The size of the test tank was not specified, so it is unclear whether it was adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.			
	Metric 17:	Outcome Assessment Methodology	Uninformative	The researchers did not describe the mortality methodology. They did not describe a range-finding test. There was no rationale offered for the mortality assessment because examining mortality due to DEHP exposure was not a goal of the study.			
	Metric 18:	Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for mortality.			
Demain (+ Conformation	- / Venishle Com	- 4 1					
Domain 6: Confounding	g / Variable Cor Matria 10:	101 Confounding Variables in Test	Madium	There was no concreted differences emerge the study around that could effect outcome			
	Metric 19:	Design and Procedures	Medium	assessment, except that the authors did not report control impurities (ambient levels of DEHP).			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.			
Domain 7. Data Dragant	tation and Anal	veie					
Domain 7. Data Fresent	Metric 21:	Statistical Methods	High	The study results were presented as means with standard errors and analyzed with one- way ANOVA and Duncan's Multiple Range test as merited.			
	Metric 22:	Reporting of Data	High	The data for all outcomes were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these were adequately discussed by the study authors.			
Additional Comments:	This study investigated the effects of various levels of DEHP exposure on growth performance, blood parameters, antioxidant enzyme activities, immune responses and resistance to the Aeromonas hydrophila challenge on yellow catfish Pelteobagrus fulvidraco. This form was used to evaluate the behavioral data reported in Table 2. Behavioral assessment was not the goal of the study, methods were not described for the behavioral assessment. Data was presented in Table 1 and in section 3.1.						

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4742097 Table: 4 of 4

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Study Citation:	Yuan, L., Li, M., Meng, F., Gong, Y., Qian,	Y., Shi, G., Wang, R. (2017). Growth, blood hea	lth, antioxidant status, immune response and resistance		
	to Aeromonas hydrophila of juvenile yellow (catfish exposed to di-2-ethylhexyl phthalate (DEH	P). Comparative Biochemistry and Physiology - Part C:		
Duration:	Overall Duration: > 21 days; Exposure Durati	on: > 21 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Pelteobagrus fulvidraco; Adu	lt			
Health Outcome:	Behavioral				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	4742097				
Domain	Metric	Rating	Comments		
Overall Quali	ty Determination	Uninformative			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Duration: Overall Daration: > 21 days: Exposure Daration: > 21 days: Exposure Autority: Water, Autority: Water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Exposure Rout: Control (iters) Control (iters) Control (iters) Taxs, Species, Age: Control (iters) Realth Outcome: Control (iters) Control (iters) Domain Metric Rating Comments Domain I: Test Substance Domain Metric I: Test Substance Identify Median Chemical was identified by name: (14C) di-2-strylhexylphthalae (DEHP) Domain I: Test Substance Identify Median Comments Comments Domain I: Test Substance Identify Median Conserve was identified as New England Nuclear. Metric (Iter) Domain 2: Test Design Ketric 3: Test Substance Purity Uninformative No negative control group was reported. Metric 4: Negative Control Reporter No negative control group was reported. No negative control group was reported. Metric 5: Reporter Metric 6: Randomized Allocation Low No negative control group was reported. Domain 3: Exposure Chur-teretrizion Metric 6: Ran	Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.						
Trans. Species. Age: Vertebrat: Fish: Ploximup ploximus: Not Applicable (e.g., fungi or algue studies) or Not Reported Pach Outcome: Different is in Ploximup ploximus: Not Applicable (e.g., fungi or algue studies) or Not Reported Demical: Different is in Ploximup ploximus: Not Applicable (e.g., fungi or algue studies) or Not Reported Domain 1: Test Substance Metric 1: Test Substance Identity Medium The chemical was identified by name: (IAC)-fi-2-chylhexylphthalate (DEHP). No CASEN or structure wave reported. Domain 1: Test Substance Source High The chemical was identified as New Fagland Nuclear. Domain 2: Test Design Metric 5: Regative Control Response N/A No negative control group was reported. Metric 5: Regative Control Response N/A No negative control group was reported. Domain 3: Exposure (Fair-Fair-Fair-Fair-Fair-Fair-Fair-Fair-	Duration: Exposure Route, Media. Path:	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Medium The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were reported. Metric 2: Test Substance Source High The source was identified as New England Nuclear. Domain 2: Test Design Metric 4: Negative Controls NiA No negative control group was reported. Metric 5: Negative Control Response N/A No negative control group was reported. Metric 6: Randomized Allocation Low No negative control group was reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 7: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 8: Consistency of Exposure High The test substance was measured by TLC and liquid similiation. Final concentrations of sediment, gass walls, surface micrology servers, and ministration or sediment was wassented by TLC and liquid similiation. Final concentrations of sediment, gass walls, surface micrology server calculated. The mass bulance was DEBEP + meabulates (Matric 1.4) ang/L	Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F ADME (biot Di-ethylhexy 59542	Fish; <i>Phoxinus phoxinus</i> ; Not Applicable (e.g transformation) yl phthalate (DEHP)	., fungi or algae studies)	or Not Reported				
Domain 1: Test Substance Metric 1: Test Substance Identity Medium The chemical was identified by name: (14C)-di-2-ehylhexylphthalate (DEHP). No CASRN or structure were reported. Metric 2: Test Substance Purity High The source was identified as New England Nuclear. Domain 2: Test Design Metric 3: Negative Controls Uninformative No negative control group was reported. Metric 5: Negative Control Response N/A No negative control group was reported. Metric 6: Randomized Allocation Low No regative control group was reported. Domain 3: Exposure Characterization Metric 6: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 9: Measurement of Test Substance High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in actione prior to adding to the system. Metric 9: Measurement of Test Substance High The st substance was neasured by duty. Must Assistantian and groups or organisms were completed at the end of system. Metric 10: Exposure Duration and Frequency High The st 2-day exposure was subleace was adjuined allocation Metric 11: Number of Exposure Levels N/A One exposure concentrati	Domain		Metric	Rating	Comments				
Metric 2: Test Substance Source High The source was identified as New England Nuclear. Domain 2: Test Substance Purity High The purity was 99.5%. Domain 2: Test Substance Purity No negative control group was reported. Metric 5: Negative Controls N/A No negative control group was reported. Metric 6: Randomized Allocation Low No random allocation was reported. Domain 3: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Preparation Metric 7: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 9: Metric 8: Consistency of Exposure High The substance was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Metric 9: Measurement of Test Substance High The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suppended material, and groups of organisms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride). Metric 10: Exposure Corups/ N/A One exposure was sufficient. Metric 11:	Domain 1: Test Substand	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were reported.				
Domain 2: Test Design Metric 4: Negative Controls Mesponse Uninformative No negative control group was reported. Metric 5: Readomized Allocation N/A No negative control group was reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Metric 6: Robuster Consistency of Exposure Administration Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 9: Consistency of Exposure Administration Metric 9: Metric 9: Concentration Metric 9: Measurement of Test Substance Concentration High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system. Metric 9: Measurement of Test Substance Concentration High The test substance was measured by TLC and liquid scintillation. Final concentrations insis were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid) and phthalic and/ydrid). Metric 11: Number of Exposure Coursel N/A One exposure concentration was utilized in this study. Spacing of Exposure Levels Medium DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility water (-1.23 mg/L). Use of acetone as a solvent may increase solubility slightly. Domain 4: Test Organism Test Organism Characteristic		Metric 2: Metric 3:	Test Substance Source Test Substance Purity	High High	The source was identified as New England Nuclear. The purity was 99.5%.				
Metric 4: Metric 5: Negative Controls Uninformative N/A No negative control group was reported. No negative control group was reported. N/A No negative control group was reported. Domain 3: Exposure Characterization Ketric 7: Experimental System/Test Media Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 8: Consistency of Exposure High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP Administration Metric 9: Measurement of Test Substance Concentration High The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride). Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: N/A One exposure concentration was utilized in this study. Domain 4: Test Organism Test Organism Characteristics Number of Organisms and Retric 13: Test Organism Characteristics Number of Organisms and Retric 13: Low Organisms were collected in the field. Age and sex were not provided in the study. Domain 4: Test Organism Kertic 13: Test Organism and Replicates per Group Low No replicates were report	Domain 2: Test Design								
Metric 5: Negative Control Response N/A No negative control group was reported. Metric 6: Randomized Allocation Low No random allocation was reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 8: Consistency of Exposure High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Administration Metric 9: Measurement of Test Substance High The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride). Metric 10: Exposure Duration and Frequency High The 27-day exposure was sufficient. Metric 12: Testing at or Below Solubility Limit Medium DEHP was dissolved in was utilized in this study. Spacing of Exposure Levels Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Domain 4: Test Organism Number of Organisms and Replicates per Group Low No replicates were reported.	6	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.				
Metric 6:Randomized AllocationLowNo random allocation was reported.Domain 3: Exposure CharacterizationMetric 7:Experimental System/Test Media PreparationMediumBiomass loading and placement of organisms in the 20L tank were not reported. PreparationMetric 8:Consistency of Exposure Administration Metric 9:HighA single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system. The test substance was measured by TLC and liquid scintillation. Final concentration of sediment Jages walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride). Metric 10:Metric 10:Exposure Duration and Frequency Spacing of Exposure Groups/ N/AMediumDomain 4: Test OrganismMetric 13:Metric 13:Test Organism Characteristics Netric 14:LowMetric 13:Test Organism Characteristics Number of Organism and Pretreatment LowLowMetric 14:Acclimatization and Pretreatment LowLowMetric 15:Test Organism Characteristics Number of Organisms and Replicates per GroupLowMetric 15:Test Organism and Pretreatment Acclimatization Number of Organisms and Number of Organisms and <b< td=""><td></td><td>Metric 5:</td><td>Negative Control Response</td><td>N/A</td><td>No negative control group was reported.</td></b<>		Metric 5:	Negative Control Response	N/A	No negative control group was reported.				
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium Biomass loading and placement of organisms in the 20L tank were not reported. Metric 8: Consistency of Exposure Administration High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Administration Metric 9: Measurement of Test Substance Concentration High The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride). Metric 10: Exposure Duration and Frequency High The 27-day exposure was sufficient. Metric 11: Number of Exposure Groups/ N/A One exposure concentration was utilized in this study. Spacing of Exposure Levels Medium DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility is within water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly. Domain 4: Test Organism Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 1		Metric 6:	Randomized Allocation	Low	No random allocation was reported.				
Metric 7:Experimental System/Test Media PreparationMediumBiomass loading and placement of organisms in the 20L tank were not reported. PreparationMetric 8:Consistency of Exposure Administration Metric 9:HighA single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system.Metric 9:Measurement of Test Substance ConcentrationHighThe test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).Metric 10:Exposure Duration and Frequency Spacing of Exposure Groups/ Spacing of Exposure Levels Metric 12:MighThe 27-day exposure concentration was utilized in this study. Spacing of Exposure Levels Metric 12:Domain 4: Test OrganismMetric 13:Test Organism Characteristics Number of Organisms and Metric 14:LowOrganisms were collected in the field. Age and sex were not provided in the study. The study did not report acclimation or pretreatment. Conditions Number of Organisms and Metric 15:LowOrganisms were reported. Replicates per Group	Domain 3: Exposure Cha	aracterization							
Metric 8: AdministrationConsistency of Exposure AdministrationHigh HighA single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acctone prior to adding to the system.Metric 9:Measurement of Test Substance ConcentrationHighThe test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).Metric 10:Exposure Duration and Frequency Metric 11:HighThe 27-day exposure was sufficient.Metric 11:Number of Exposure Groups/ Spacing of Exposure Levels Metric 12:N/AOne exposure concentration was utilized in this study.Domain 4: Test OrganismTest Organism Characteristics Number of Organism sand Metric 14:LowOrganisms were collected in the field. Age and sex were not provided in the study. The study did not report acclimation or pretreatment. Conditions Number of Organisms and Replicates per Group	Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.				
Metric 9:Measurement of Test Substance ConcentrationHighThe test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass 		Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system.				
Metric 10: Exposure Duration and Frequency Metric 11: High Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: The 27-day exposure was sufficient. Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: N/A One exposure concentration was utilized in this study. Domain 4: Test Organism Test Organism Characteristics Medium DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility slightly. Domain 4: Test Organism Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Metric 15: Number of Organisms and Replicates per Group Low No replicates were reported.		Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).				
Metric 11:Number of Exposure Groups/N/AOne exposure concentration was utilized in this study.Metric 12:Spacing of Exposure Levels Testing at or Below Solubility LimitMediumDEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.Domain 4: Test OrganismMetric 13:Test Organism CharacteristicsLowMetric 14:Acclimatization and PretreatmentLowOrganisms were collected in the field. Age and sex were not provided in the study.Metric 15:Conditions Number of Organisms and Replicates per GroupLowNo replicates were reported.		Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.				
Metric 12: Testing at or Below Solubility Limit Medium DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Metric 15: Number of Organisms and Replicates per Group Low No replicates were reported.		Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One exposure concentration was utilized in this study.				
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Metric 15: Conditions Number of Organisms and Replicates per Group Low No replicates were reported.		Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.				
Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Metric 15: Number of Organisms and Low No replicates were reported. Metric 15: Replicates per Group Low No replicates were reported.	Domain 4: Test Organisi	n							
Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Conditions Number of Organisms and Low No replicates were reported. Metric 15: Replicates per Group Conditions No replicates were reported.	0.1	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.				
Conditions Metric 15: Conditions Number of Organisms and Replicates per Group Low No replicates were reported.		Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.				
Replicates per Group		Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.				
			Replicates per Group						

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		co	ntinued from previou	s page				
Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.						
Duration:	Overall Dur	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Vertebrate; I	Fish; Phoxinus phoxinus; Not Applicable (e.g.	, fungi or algae studies) or Not Reported				
Health Outcome:	ADME (bio	transformation)						
Chemical: HERO ID:	59542	yl phthalate (DEHP)						
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of feed for fish were not well described.				
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).				
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.				
Domain 6: Confounding	g / Variable Co	ntrol						
·	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP uptake by each organism.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.				
Domain 7: Data Present	tation and Anal	lysis						
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.				
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.				
Additional Comments:	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites, the metabolites being phthalic acid and phthalic anhydride.							
Overall Quali	ty Deterr	nination	Uninformativ	7e				

Study Citation:	Adams, W. J	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic						
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	Dverall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate: F	Fish: Pimephales promelas: Juvenile						
Health Outcome:	Mortality	,						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	1321996							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.				
	Metric 2:	Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.				
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.				
Domain 2: Test Design								
2 onium 21 1000 2 001gn	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5:	Negative Control Response	High	The control response was acceptable.				
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.				
	Metric 9:	Administration Measurement of Test Substance	High	Sample extracts were analyzed by gas chromatography at the start and the end of the				
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.				
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.				
Domain 4: Test Organisi	n							
2 onium 1. rost organisi	Metric 13:	Test Organism Characteristics	Low	The source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.				
	Metric 15:	Conditions Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test				
		Replicates per Group		vessel.				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.				
Continued on next page								

		conti	nued from p	revious page		
Study Citation:	Adams, W. J	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic				
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-1	574.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Pimephales promelas; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.		
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent across groups.		
	Assessment					
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	The environmental conditions were consistent across groups.		
		Design and Procedures	e e			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.		
Domain /: Data Present	ation and Anal	ysis	TT: 1			
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.		
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					
Overall Quality Determination		High				

	Continued on next page							
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.				
Domain 5: Outcome As	Sessment Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test				
		Replicates per Group		vessel.				
	Metric 15:	Conditions Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.				
Domain 4: Test Organis	m							
	mente 12.	resting at or below Solubility Ellilit	mgn	The test was performed at of below the water solubility lillint.				
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit				
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.				
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of exposure were appropriate for the test.				
		Concentration		test. In static studies, final test concentrations frequently were 50% of the initial concen- trations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.				
	Metric 9:	Administration Measurement of Test Substance	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the				
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 6:	Kanuoinizeu Anocation	LOW	The anocation method was not reported.				
	Metric 5: Matric 6:	Negative Control Response	High	The control response was acceptable.				
	Metric 4:	Negative Controls	High	A negative control was reported.				
Domain 2: Test Design								
	incure 5.		mgn					
	Metric 3:	Test Substance Purity	High	was reported. There was at least 95% purity.				
	Metric 2:	Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data				
1000 0 0000tull	Metric 1:	Test Substance Identity	Low	Test substance nomenclature was reported without CASRN.				
Domain 1: Test Substan	ce	Metric	Katilig	Comments				
Domain		Metric	Pating	Comments				
HERO ID:	1321996	1321996						
Health Outcome:	Mortality							
Taxa, Species, Age:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile							
Media, Path:								
Exposure Route,	Aquatic (fres	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)						
Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic							

		conti	nued from p	revious page		
Study Citation:	Adams, W. J	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic				
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-15	574.		
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (fres	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	ish; Pimephales promelas; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent across groups.		
		Assessment				
Domain 6: Confounding	v / Variable Cor	atrol				
Domain 0. Comounding	Metric 10	Confounding Variables in Test	High	The anyironmental conditions were consistent across groups		
	Wieute 19.	Design and Procedures	Ingn	The environmental conditions were consistent across groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.		
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					
Overall Quality Determination		High				

Study Citation: Duration: Exposure Route, Media Path:	 Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake 						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 1316188	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP) 1316188					
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by an accepted name [di(2- ethylhexyl) phthalate; DEHP].			
	Metric 2:	Test Substance Source	Low	The study states "The fourteen phthalate esters, all clear colorless liquids, labeled IA through IN, were received on 18 December 1981 in 1-liter amber glass bottles."In the appendix on analytical methods, the study states that the test chemicals were "supplied by the Chemical ManufacturersAssociation, Washington. D.C."However, specific details about the test substance source (manufacturer, batch/lot #, etc.,) were not reported, and test substance identities were not analytically verified.			
	Metric 3:	Test Substance Purity	Low	Purities of the test substances were not reported.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Appropriate, concurrent negative control groups were used –"Duplicate control jars containing the same dilution water andmaintained under the same conditions as the			
	Metric 5:	Negative Control Response	High	exposure jars, butcontaining no test material, were established." No mortalities were observed in the control groups, and the study notes that "the pH val- ues and dissolved oxygen concentrations remained comparable to the respective controls during exposures to solutions of phthalate esters IE through IN [the test substances."			
	Metric 6:	Randomized Allocation	Medium	"Ten fathead minnows (population descriptions in Table 1) wererandomly distributed to each test jar after the test solutionshad been prepared."			
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	Experimental system and test media preparation were described in adequate detail. Tests were conducted in 19.6L glass jars, filled with 15L of dilution water and appropriate amount of test substance to achieve the desired concentration. Temperature was controlled. "Dilution water used was soft water reconstituted from deionized water" (characteristics given); test solutions were not aerated. Water temperature, pH, DO, hardness, alkalinity, specific conductance were all monitored. No measures were taken to prevent loss of test substance over the course of the exposure, and in fact the authors reported that "an appreciable loss of phthalate ester from solution occurred during each exposure. The concentrations of phthalate ester present in solution at the end of the exposures ranged from <4 to 68% of the 0-hour concentration."			
Continued on next page							

Diethylhexyl Phthalate

Environmental Hazard Evaluation

	continued from previous page
Study Citation:	Bionomics, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	1316188

Domain	Metric	Rating	Comments
Metric 8:	Consistency of Exposure Administration	Low	Since no measures were used to maintain a consistent exposure concentration over the course of the experiment, there is no way to know how confidently two different exposure groups can be compared. The 96hr concentration shows the minimum possible exposure the test organisms were subjected to, but since no intermediate measurements were reported, the rate at which the test substance was lost, and how consistently the loss occurred between groups is unknown.
Metric 9:	Measurement of Test Substance Concentration	Medium	Test substance concentrations were only measured at the beginning and end of the study (0-hr and 96-hr). Analytical methods were appropriate, and they were detailed in an appendix – "An aliquot of the concentrate was analyzed by gas-liquidchromatography with electron capture detection."
Metric 10:	Exposure Duration and Frequency	High	The exposure duration is in accordance with the cited methodology [Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).]
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	For "corroborative tests" (tests for chemicals which showed no acute toxicity at their limit of solubility), only one exposure concentration was tested. The test substances within the limit of solubility were tested in triplicate.
Metric 12:	Testing at or Below Solubility Limit	High	No exposure concentrations were above the limit of solubility; as such, no solvents were necessary.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	Complete details regarding test organism characteristics are lacking. Only the source ("fathead minnows were obtained from cultures maintained at EG&G, Bionomics, Wareham, Massachusetts") and the mean length and mean weight of the test organisms in each fish population lot were reported.
Metric 14:	Acclimatization and Pretreatment Conditions	High	All test organisms underwent the same pretreatment process, including a 14-day pre- treatment period and a 48-hr acclimatization period immediately before the tests (de- scribed in detail on page 2 of the study).
Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish were included in each treatment group, and for the corroborative tests, three replicates were conducted for each test substance.
Domain 5: Outcome Assessment Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate – well detailed on pages 2-4 of the study (16hr light /8hr dark photoperiod; dry food fed ad libitum during the pretreatment period; 22+/-1 °c temp; measured water parameters stated).
	Cont	tinued on nex	t page

		continued from previous page			
Study Citation:	Bionomics,, EG&G (1983). Acute toxicity of	fourteen phthalate esters to fathead m	innows.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	re Duration: 0 - 4 days (0-96h)			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Not A	Applicable (e.g., fungi or algae studies)) or Not Reported		
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1316188				
Domain	Metric	Rating	Comments		

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	The authors report the percentage mortality for each study group at 24hr, 48hr, 72hr and 96hr. Since repeated concentration measurements were not taken, the accuracy of a NOEC derived from this data is limited. A NOEC can be derived from the lowest reported concentration (96hr), but the actual exposure experienced by the test organ- isms could range anywhere between there and the highest reported (0hr) concentration –"Concentrations of phthalate esters measured in solution at the initiation of the expo- sures ranged from 52 to 91% of the nominal concentrations (IN and IF, respectively). An appreciable loss of phthalate ester from solution occurred during each exposure. The concentrations of phthalate ester present in solution at the end of the exposures ranged from <4 to 68% of the 0-hour concentration IE and IN, respectively)."
	Metric 18:	Consistency of Outcome Assessment	High	No significant effects occurred, though it seems that like experimental conditions, out- come assessment was carried out consistently across all study groups."No significant effects were observed among fathead minnows exposed to a single concentration of ph- thalate esters IE through IN representative of each materials limit of water solubility"
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Many variables were measured, and appear to be consistent across study and control groups – "Good quality control was maintained throughout theanalyses as indicated by the QA spiked sample analytical results. The pH values and dissolved oxygen concentrations remained comparable to the respective controls during exposures to solutions of phthalate esters IE through IN."
	Metric 20:	Outcomes Unrelated to Exposure	High	No attrition of test organisms unrelated to the exposure was reported.
Domain 7: Data Process	otion and Arr-1			
Domain 7: Data Present	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary (results presented were just percentage mortality, at 0%).
	Metric 22:	Reporting of Data	High	Data is reported for all study groups in the appendix.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes occurred.
Additional Comments:	None			
Overall Qualit	ty Detern	nination	High	

Study Citation: Duration: Exposure Route, Media Path:	Bionomics,, I Overall Dura Aquatic (fres	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP) 1316189							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		_					
	Metric 1:	Test Substance Identity	Low	The test substance (Diisooctyl phthalate; isomer of DEHP) was identified only by nomenclature. No other information (CASRN, structure, etc.) was provided.				
	Metric 2:	Test Substance Source	Low	The test substance was received from the General Electric Company, Hudson Falls, New York, on 11 and 18 December 1981. The test substance identity was NOT analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	The test substance was reported as"100% active ingredient." It is doubtful that it is lit- erally one hundred percent, but this can be taken to mean very pure as received from manufacturer.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Negative controls were used.				
	Metric 5:	Negative Control Response	High	There was no unacceptable mortality in controls.				
	Metric 6:	Randomized Allocation	Medium	"The test was initiated when ten fathead minnows were randomly distributed to each aquarium "				
Domain 3: Exposure Ch	aracterization							
-	Metric 7:	Experimental System/Test Media Preparation	High	The flow-through regime was described in detail. Stock solutions were prepared daily & were maintained throughout experiment.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	High	Test substance concentrations were measured pre-test and at 0h and 96h.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure and/or the exposure frequency were reported and appropri- ate for the study type and/or outcome(s) of interest (96-h acute toxicity study for fathead minnows, extended to 144h according to study design).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number and the spacing of exposure groups were adequate.				
	Metric 12:	Testing at or Below Solubility Limit	High	The highest dose chosen was at the solubility limit of the chemical as "communicated verbally to EG&G Bionomics from the Syracuse Research Corporation (1982)" – though some droplets of undissolved phthalate were seen at high concentrations. See Appendix I for details of the solubilizing apparatus used to disperse phthalates in exposure water.				

Domain 4: Test Organism

Diethylhexyl Phthalate

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Overall Dura Aquatic (free Vertebrate; F Mortality Di-ethylhexy 1316189	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP) 1316189				
Domain		Metric	Rating	Comments		
	Metric 13:	Test Organism Characteristics	Medium	Fathead minnows were from in-house cultures. Length and wet weight measurements were given in Table 1.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The acclimation was performed in holding tanks for "a minimum of 14 days" for both control & exposed fish.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates.		
Domain 5: Outcome As	ssessment					
	Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	High High High	Test conditions were described in detail & were adequate for the health of P. promelas. Fish were observed for mortality every 24 hours. Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in attrition or other health outcomes.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	No mortality was observed in any treatment group. Statistical analysis was not neces- sary.		
	Metric 22:	Reporting of Data	High	Data for all outcomes was presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	: This evaluation was for the non-definitive 96hr LC 50 value reported for DIOP.					

Overall Quality Determination

High

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Study Citation: Duration: Exposure Route, Media, Path:	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Di-ethylhexy 1316189	Fish; <i>Pimephales promelas</i> ; Not Applicable	e (e.g., fungi e	or algae studies) or Not Reported	
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure, etc.) was provided.	
	Metric 2:	Test Substance Source	Low	The DEHP was received from the General Electric Company, Hudson Falls, New York, on 11 and 18 December 1981. The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	"100% active ingredient" was reported. It is doubtful that it is literally one hundred percent, but this can be taken to mean very pure as received from the manufacturer.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative controls were used.	
	Metric 5:	Negative Control Response	High	There was no unacceptable mortality in the controls.	
	Metric 6:	Randomized Allocation	Medium	"The test was initiated when ten fathead minnows were randomly distributed to each aquarium "	
Domain 3: Exposure Ch	aracterization				
Ĩ	Metric 7:	Experimental System/Test Media Preparation	High	The flow-through regime was described in detail. Stock solutions were prepared daily & were maintained throughout the experiment.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Administration Measurement of Test Substance	High	Test substance concentrations were measured pre-test and at 0h and 96h.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure and/or the exposure frequency were reported and appropri- ate for the study type and/or outcome(s) of interest (96-h acute toxicity study for fathead minnows, extended to 144h according to study design).	
	Metric 11:	Number of Exposure Groups/	High	The number and the spacing of exposure groups were adequate.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The highest dose chosen was at the solubility limit of the chemical as "communicated verbally to EG&G Bionomics from the Syracuse Research Corporation (1982)" – though some droplets of undissolved phthalate were seen at high concentrations. See Appendix I for details of the solubilizing apparatus used to disperse phthalates in exposure water.	
Domain 4: Test Organis	m				
2 shian in fost organis.	Metric 13:	Test Organism Characteristics	Medium	Fathead minnows were from an in-house culture. Length and wet weight measurements were given in Table 1.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation was performed in holding tanks for "a minimum of 14 days" for both con- trol & exposed fish.	
Continued on next page					

Diethylhexyl Phthalate

HERO ID: 1316189 Table: 2 of 2

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Bionomics,, I Overall Dura Aquatic (fres Vertebrate; F Mortality Di-ethylhexy	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality					
HERO ID:	1316189	1316189					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were described in detail & adequate for the health of P. promelas.			
	Metric 17:	Outcome Assessment Methodology	High	Fish were observed for mortality every 24 hours.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	/ Variable Con	trol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in attrition or other health outcomes.			
Domain 7: Data Presenta	ation and Analy	ysis					
	Metric 21:	Statistical Methods	N/A	No mortality was observed in any treatment group. Statistical analysis was not neces- sary.			
	Metric 22:	Reporting of Data	High	Data for all outcomes was presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	This evaluati	This evaluation is for the non-definitive 96hr LC 50 value reported for DEHP.					

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 5774391				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	Low	The full chemical name is provided in addition to the acronym, but no specific CAS number or additional information is provided.	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified.	
	Metric 3:	Test Substance Purity	High	The DEHP was high-purity (>99%) from a commercial source.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No	
		C C	C	solvents were used to necessitate a solvent control.	
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with the control groups.	
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for mea- surements.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as the develop- ment of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.	
	Metric 8:	Consistency of Exposure	High	Details of the exposure are provided and are consistent among study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.	
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations allowed for calculation of an LC50.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentra- tions was provided, and care was taken to ensure minimal degradation or loss of the test substance during experiments.	
Domain 4: Test Organisi	n				
2	Metric 13:	Test Organism Characteristics	High	Test organisms were obtained from a reliable source and test organism details were provided.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.	
Continued on next page					

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5774391 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Defoe, D. L. Environment	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environmental Toxicology and Chemistry 9(5):623-636					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Pimephales promelas; Juvenile					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome of interest (LC50) was appropriate.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment	-				
Domain 6: Confounding	g / Variable Cor	ntrol					
c	Metric 19:	Confounding Variables in Test	High	There were no variations or inconsistencies reported across study groups, and environ-			
		Design and Procedures	C	mental conditions are provided.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors provided discussion on different results measured in other papers.			
Additional Comments:	DEHP was not acutely toxic to fathead minnows at the highest tested concentration (above water solubility). Control, medium, and high test concentration treatments were reported. The LC50 was calculated, but minimal raw data was provided.						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i>; Embryo Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 2071071 				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.	
	Metric 2:	Test Substance Source	Low	The source was not reported.	
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.	
Domain 2: Test Design	Matria 4:	Nagativa Controls	High	Authors reported using both a water control and a corrier control (0.001.0% others leads)	
	Meuric 4.	Negative Controls	nigii	tion).	
	Metric 5:	Negative Control Response	Medium	The authors reported the genomic methylation levels for the control group, which are believed to be adequate. However, the authors did not report the specific mRNA levels for the gene expression analysis.	
	Metric 6:	Randomized Allocation	Low	The allocation method was not detailed as random allocation.	
Domain 3: Exposure Ch	aracterization				
	Metric /:	Experimental System/Test Media Preparation	Medium	Details in the preparation of the solutions for the embryonic DEHP experiment were lacking. But the authors did indicate that the exposure was carried out for 48 h at ambient temperature, and the chemicals were prepared fresh and renewed at 24 h with a 90 % water change.	
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatment and control groups.	
	Metric 9:	Measurement of Test Substance	Low	There was no measurement of treatment concentrations. All concentrations were pre- sented as nominal.	
	Metric 10:	Exposure Duration and Frequency	High	The goal was to assess dmnt expression in embryos (pre-hatch), therefore a 48-hour exposure was appropriate.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).	
	Metric 12:	Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.	

Domain 4: Test Organism

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 3071071 Table: 1 of 2

		contin	ued from previ	ous page		
Study Citation: Duration: Exposure Route, Modia Path:	 Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathe (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptak 					
Taxa, Species, Age: Health Outcome:	Vertebrate; l Mechanistic	Fish; <i>Pimephales promelas</i> ; Embryo -Cell signaling/function				
HERO ID:	3071071	yi phinalate (DEHP)				
Domain		Metric	Rating	Comments		
	Metric 13:	Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors reported that embryos were evenly distributed into 12 glass petri dishes per treatment group, and that at 24 h, up to 3 embryos per replicate were collected. How- ever, there is no clear indication of the total number of organisms (embryos) distributed to each of the 12 petri dishes.		
Domain 5: Outcome A	Assessment					
	Metric 16:	Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (RNA extraction and real-time PCR) was clearly described for the intended outcome of interest (measuring expression of the dnmt genes to understand epigenetic mechanisms).		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment and control groups.		
Domain 6: Confoundi	ng / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influ- enced the outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.		
Domain 7: Data Prese	ntation and Ana	lysis				
	Metric 21:	Statistical Methods	High	Authors used ANOVA and Tukey's post-hoc for gene expression. Normality and homo- geneity of variance were tested with Shapiro-Wilks and Levene's test, respectively.		
	Metric 22:	Reporting of Data	Low	Embryo experiment: The expression of dnmt per treatment group was not reported. Results were not shown in a table or figure. In the text, the authors reported that following a 24-h exposure, no differences were detected among groups for mRNA levels of dnmt1 (df = 3; H = 1.5; p = 0.68), dnmt3 (H = 3.9; p = 0.28), dnmt6 (H = 3.5; p = 0.33), dnmt7 (H = 3.5; p = 0.34), or dnmt8 (H = 0.51; p = 0.92).		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 3071071 Table: 1 of 2

continued from previous page						
Study Citation:	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposur	re Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Water; Not determined l	by study authors (i.e., chemical of intere	est in exposure water, but unable to determine exact uptake route)			
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Embry	уо				
Health Outcome:	Mechanistic-Cell signaling/function					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3071071					
Domain	Metric	Rating	Comments			
Additional Comments:	The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the					
	expression of the dnmt genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate embryo expression of					
	the dnmt genes. The authors did not assess glo	obal DNA methylation for the embryos.				

Overall Quality Determination

Medium

Duration: Exposure Route, Media, Path:	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; F	Fish; Pimephales promelas; Embryo				
Health Outcome:	Mortality					
Chemical: HERO ID:	Di-ethylhexy 3071071	/l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).		
	Metric 5:	Negative Control Response	Low	The authors did not report the actual survival rate of the control groups (water control or carrier/solvent control). The only information they offered about the embryo mortality was "In the first experiment, a 48-h exposure to one of three concentrations of phthalates did not result in any significant mortality compared to the controls ($H = 6.16$; $p = 0.10$)."		
	Metric 6:	Randomized Allocation	Low	The embryo exposure was not detailed as random allocation.		
Domain 3: Exposure Cha	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	Details in the preparation of the solutions for the embryonic DEHP experiment was lacking. But the authors did indicate that the exposure was carried out for 48 h at ambient temperature, and the chemicals were prepared fresh and were renewed at 24 h with a 90 % water change.		
	Metric 8:	Consistency of Exposure	High	Exposure administration appeared consistent among treatment and control groups.		
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were pre- sented as nominal (1, 10, and 100 ug/L).		
	Metric 10:	Exposure Duration and Frequency	High	This goal was to assess mortality in embryos (pre-hatch), therefore a 48-hour exposure was appropriate.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).		
	Metric 12:	Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.		

Diethylhexyl Phthalate

		contin	ued from previ	ous page				
Study Citation: Duration:	Wood, R. K (Pimephales Overall Dura	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Media, Path:	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo Mortality Di-ethylhexyl phthalate (DEHP) 3071071							
Domain		Metric	Rating	Comments				
	Metric 13:	Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors reported that embryos were evenly distributed into 12 glass petri dishes per treatment group, and at 24 h, up to 3 embryos per replicate were collected. However, there is no clear indication of the total number of organisms (embryos) distributed to each of the 12 petri dishes.				
Domain 5: Outcome A	ssessment							
	Metric 16:	Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (counts of dead organisms) was not clearly de- scribed for the intended outcome of interest (mortality due to DEHP exposure). Briefly, the authors reported that the exposure was carried out for 48 h, and at 24 h, up to 3 indi- viduals per replicate were collected, and at 48 h, the number of surviving embryos was assessed in each treatment. Unfortunately, there is not clear indication of whether dead organisms were counted at 24h or if that only happened at 48 h.				
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment and control groups.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influ- enced the outcome assessment.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.				
Domain 7: Data Presen	tation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Authors reported that differences in survival and hatch were tested using a Mantel–Cox log-rank test.				
	Metric 22:	Reporting of Data	Low	Enbryo experiment: The mortality rate per treatment group was not reported. Results were not shown in a table or figure. In the text, the authors reported that a 48-h exposure to one of three concentrations of DEHP did not result in any significant mortality compared to the controls (H = 6.16 ; p = 0.10).				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.				

High

Diethylhexyl Phthalate

continued from previous page					
Study Citation:	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow				
D	(Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Embryo				
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	3071071				
Domain	Metric	Rating	Comments		
Additional Comments:	The objectives of this study were to determine wexpression of the dnmt genes and in vivo DNA m	vhether DEHP exposure affects ethylation in fathead minnow en	survival and development via epigenetic mechanisms by measuring the abryos and larvae. This form was used to evaluate embryo mortality.		

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae Mortality Di-ethylhexyl phthalate (DEHP) 3071071					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.		
Domain 2: Test Design	Metric 4:	Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solu-		
		C	C	tion).		
	Metric 5:	Negative Control Response	Medium	Cumulative mortality for the 14 day exposure in control groups was approximately 20%. This is not an acute test (10% requirement) and larval survival (80%) over this period is not unexpected.		
	Metric 6:	Randomized Allocation	Medium	The authors reported: "Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate."		
Domain 2. Euroques Ch	anaatanizatian					
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The authors offered details on on using the stock solution to prepare the exposure solutions every 48 hours: the control water (ethanol added), theD1water (DEHP dissolved in ethanol added to make a 1.0-ug/L solution), the D2 water (DEHP dissolved in ethanol added to make a 10.0-ug/L solution), and the D3 water (DEHP dissolved in ethanol to make a 100.0-ug/L solution).		
	Metric 8:	Consistency of Exposure	High	Exposure administration appeared consistent among treatment and control groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were pre- sented as nominal.		
	Metric 10:	Exposure Duration and Frequency	High	Larval study: The study examined endpoints for mortality throughout 14 days in larvae [0 days post-hatch (dph)]. The authors explained that this life stage was chosen for the exposure because research conducted in zebrafish showed that there is high dnmt expression in the post-hatch stage (Smith et al. 2011), and investigating DNA methylation patterns was the main goal of the study.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).		
Continued on next page						

Diethylhexyl Phthalate

	continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i>; Larvae Mortality Di-ethylhexyl phthalate (DEHP) 3071071 					
Domain		Metric	Rating	Comments		
	Metric 12:	Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.		
Domain 4: Test Organis	sm					
	Metric 13:	Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Laval experiment: The organisms were placed in 40 glass petri dishes arranged into 4 groups of 10. Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate. The 4 treatments were a carrier control, ($n = 10$), 1 lg/L DEHP ($n = 10$), 10 lg/L DEHP, ($n = 10$), and 100 lg/L DEHP ($n = 10$).		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (counts of dead organisms) was clearly described and appropriate for the intended outcome of interest (mortality due to DEHP exposure).		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported with criteria for mortality and assessed the same across treatment and control groups. Briefly, the authors reported that each replicate was checked daily for mortalities (completely white body and/or lack of a heartbeat under a dissection microscope). Mortalities were recorded each morning be- fore the water was changed, and the exposure was carried out for fourteen days post- hatch (*19–20 days total postfertilization).		
Domain 6: Confoundin	o / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	Nothing was presented to indicate that environmental conditions or other factors influ- enced the outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.		

Domain 7: Data Presentation and Analysis

Metric 21:	Statistical Methods	High	"Differences in survival and hatch were tested using aMantel–Cox log-rank test."
Metric 22:	Reporting of Data	High	Percent cumulative mortality is presented in Figure 2.
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Diethylhexyl Phthalate

	continued from previous page				
Study Citation:	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow				
Duration:	(Pimephales prometas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1): /-18. Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Larvae				
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	3071071				
Domain	Metric Rating Comments				
	Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported.				
Additional Comments:	The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the dnmt genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate mortality in larvae.				

Overall Quality Determination

High

Study Citation:	Wood P K Crowley F Martyniuk C I (20)15) Developmental profiles	and expression of the DNA methyltransferase genes in the fathead minnow					
Study Citation.	(Pimephales promelas) following exposure to di	(Pimenbales promelas) following exposure to di 2-ethylbevyl phthalate. Eish Physiology and Biochemistry 42(1):7-18						
Duration:	Overall Duration: 11 - 21 days; Exposure Durat	ion: 11 - 21 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by	study authors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:								
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Larvae							
Health Outcome:	Mechanistic-Cell signaling/function							
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	3071071							
Domain	Metric	Rating	Comments					
Domain 1: Test Substar	0.69							

Domain		Metric	Rating	Comments
Domain 1: Test Substance	æ			
	Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, structure, or other chemical descriptors.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).
	Metric 5:	Negative Control Response	Medium	Control gene expression was not explicitly detailed, however, it is incorporated into the "normalized expression" of each dnmt gene as a fold expression value.
	Metric 6:	Randomized Allocation	Medium	The authors reported: "Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate."
Domain 3: Exposure Cha	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The authors offered details on using the stock solution to prepare the exposure solutions every 48 hours: the control water (ethanol added), theD1water (DEHP dissolved in ethanol added to make a 1.0-ug/L solution), the D2 water (DEHP dissolved in ethanol added to make a 10.0-ug/L solution), and the D3 water (DEHP dissolved in ethanol to make a 100.0-ug/L solution).
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatment and control groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were pre- sented as nominal.
	Metric 10:	Exposure Duration and Frequency	High	Larval study: The study examined endpoints for mRNA expression of the dnmt genes and global DNA methylation throughout 14 days in larvae [0 days post-hatch (dph)]. The authors explained that this life stage was chosen for the exposure because research conducted in zebrafish showed that there is high dnmt expression in the post-hatch stage (Smith et al. 2011).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).
	Metric 12:	Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pimephales promelas</i>; Larvae Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 3071071 				
Domain		Metric	Rating	Comments	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).	
	Metric 14:	Acclimatization and Pretreatment	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen light cycle, temperature, and pH	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Laval experiment: The organisms were placed in 40 glass petri dishes arranged into 4 groups of 10. Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate. The 4 treatments were a carrier control, (n = 10), 1 lg/L DEHP (n = 10), 10 lg/L DEHP, (n = 10), and 100 lg/L DEHP (n = 10).	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (RNA extraction, real-time PCR, DNA extrac- tion, and global DNA methylation) was clearly described and adequate for the intended outcome of interest (measuring dnmt gene expression and DNA methylation to under- stand epigenetic mechanisms).	
	Metric 18:	Consistency of Outcome Assessment	High	The methods/techniques used were assessed consistently across study groups.	
Domain 6: Confounding	/ Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test	High	Nothing was presented to indicate that environmental conditions or other factors influ- enced the outcome assessment	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.	
Domain 7. Data Present	ation and Anal	veis			
	Metric 21:	Statistical Methods	High	Authors used ANOVA and Tukey's post-hoc to analyze gene expression and DNA methylation data. Normality and homogeneity of variance were tested with Shapiro-Wilks and Levene's test, respectively.	
	Metric 22:	Reporting of Data	Low	The authors clearly reported the DNA methylation data in the text and Figure 2. How- ever, they did not offer results of the dnmt gene expression data.	
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	

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Diethylhexyl Phthalate

... continued from previous page Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow **Study Citation:** (Pimephales promelas) following exposure to di-2-ethylhexyl phthalate. Fish Physiology and Biochemistry 42(1):7-18. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days **Duration: Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Pimephales promelas; Larvae **Health Outcome:** Mechanistic-Cell signaling/function Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 3071071 Domain Metric Rating Comments Additional Comments: The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the dnmt genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate expression of the dnmt genes and global DNA methylation in larvae.

Overall Quality Determination

High

Study Citation:	Crago, J., K minnow (Pin	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.						
Exposure Route, Media, Path:	Aquatic (fres	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; F	ish; Pimephales promelas; Adult						
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	1014765							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name as di(2-ethylhexyl) phthalate (DEHP).				
	Metric 2:	Test Substance Source	Low	The test substance was obtained from Sigma-Aldrich, but the chemical identity was not certified and not analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the test substance was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative solvent control group.				
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was adequate in that it was re- ported as not significantly different from any treatment result.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3. Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was used.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4. Test O								
Domain 4: Test Organisi	Metric 13.	Test Organism Characteristics	Medium	Age length and initial weights of test organisms were not reported				
	Metric 14	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two days				
	Matria 15.	Conditions	Madium	There ware 10 test encourisms for each test compartmetion (control or 1 the size 1 test				
	wieure 15:	Replicates per Group	weatuill	concentration). There were no replicates.				

Domain 5: Outcome Assessment

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HERO ID: 1014765 Table: 1 of 3

		conti	nued from previo	us page			
Study Citation:	Crago, J., K minnow (Pir	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Adult						
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1014765						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups.						
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical methods were adequately described in section 2.5.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluat	ion is for GSI.					
Overall Quality Determination			Medium				

Study Citation:	Crago, J., K	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead					
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (fre	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; I	Fish; Pimephales promelas; Adult					
Health Outcome:	Mechanistic	-ADME					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
	1014/05						
Domain Domain 1: Test Substan	29	Metric	Rating	Comments			
Domain 1. Test Substan	Metric 1	Test Substance Identity	High	The chemical was identified as di(2-ethylbexyl) nhthalate (DEHP)			
	Metric 2:	Test Substance Source	Low	The test substance source was Sigma-Aldrich, but the materials were not certified or analytically verified in the study.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of test substance were not reported.			
Domain 2: Test Design	Matric 1:	Negative Controls	High	Study authors reported using an appropriate consurrant pagative solvent control group			
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		TT' 1				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was used.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
U	Metric 13:	Test Organism Characteristics	Medium	Initial ages, lengths, and weights of test organisms were not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two days.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 test organisms per test concentration and no replicates.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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HERO ID: 1014765 Table: 2 of 3

		contir	nued from p	previous page				
Study Citation:	Crago, J., K minnow (Pir	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	-							
Taxa, Species, Age:	Vertebrate; I	Fish; Pimephales promelas; Adult						
Health Outcome:	Mechanistic	-ADME						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	1014765							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	High	Statistical analysis was performed and was appropriate for the dataset.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group, and results were described in the text.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	This evaluat	This evaluation is for the assessment of plasma testosterone and 17beta-estradiol following exposure to DEHP.						
Overall Qualit	ty Deterr	nination	High					

Study Citation:	Crago, J., K minnow (Pir	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.					
Duration: Exposure Route, Madia Datha	Overall Dura Aquatic (free	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path: Taxa, Species, Age:	Vertebrate: F	Vertebrate: Fish: <i>Pimenhales promelas</i> : Adult					
Health Outcome:	Mechanistic	-Cell signaling/function-Genotox (includir	ng DNA repai	r)			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1014765						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT' 1				
	Metric 1:	Test Substance Identity	High	The chemical was identified as di(2-ethylhexyl) phthalate (DEHP).			
	Meuric 2.	Test Substance Source	LOW	analytically verified in the study.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative solvent control group.			
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Initial ages, lengths, and weights of the test organisms were not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two days.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 10 test organisms per test concentration and no replicates.			
Demain 5: Outer A		· · ·					
Domain 5: Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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Diethylhexyl Phthalate

	continued from previous page					
Study Citation:	Crago, J., K minnow (Pir	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead ninnow (Pimephales promelas) through a novel mechanism of action. Aquatic Toxicology 110-111:74-83.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; I	Fish; Pimephales promelas; Adult				
Health Outcome:	Mechanistic	-Cell signaling/function-Genotox (including	g DNA repa	ir)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1014765					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
	Assessment					
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures	C			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data 1 1050m	Metric 21	Statistical Methods	High	Statistical analysis was performed and was appropriate for dataset		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group, and		
	Metric 22.	Reporting of Data	mgn	results were described in the text.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quality Determination Hig			High			

Study Citation:	Mehrle, P. N	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace				
Duration	Substances i	Substances in Environmental Health 10:519-524. Overall Duration: > 21 days: Exposure Duration: > 21 days				
Exposure Route.	Aquatic (fre	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	riquitie (iie	sitvater), water, not determined by study ad		near of interest in exposate water, but anable to determine exact aptake router		
Taxa, Species, Age:	Vertebrate: I	Fish: Pimenhales promelas: Adult				
Health Outcome:	ADME (bio	transformation)				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	791717	(DDIII)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the test substance were not reported.		
Domain 2: Test Design	Metric 4.	Negative Controls	Low	It was unclear if the author used a negative or a solvent control		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
	incure o.	Rundonii Zou Thiobulion	Low			
Domain 3: Exposure Ch	naracterization					
· · · · · ·	Metric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-		
		Preparation	8	scribed in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered		
		Administration		consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Researchers used a radio labelled chemical to monitor concentrations.		
	Matria 10.	Concentration	II: -h			
	Metric 10:	Exposure Duration and Frequency	High	A flow-through design with an appropriate duration were reported.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.		
Domain 4: Test Organis	m					
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized		
		Conditions	2011			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (3) were reported and sufficient to charac-		
		Replicates per Group		terize toxicological effects.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health		
	mente 10.	racquies of rest conditions	meanum	though few details were provided.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.		
		Contin	ued on next pa	ge		

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Environmental Hazard Evaluation

HERO ID: 791717 Table: 1 of 3

		continu	ued from previ	ious page			
Study Citation:	Mehrle, P. N	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace					
	Substances i	Substances in Environmental Health 10:519-524.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	lays				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Pimephales promelas; Adult						
Health Outcome:	ADME (biot	transformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	791717						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
Domain 6: Confounding	y / Variable Co	ntrol					
Domain of Comountaing	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	U				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	veis					
Domain 7. Data Present	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted, which is typical for BCF assessments.			
	Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes, not for the controls.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Quali	ty Detern	nination	Medium				

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Substances in Environmental Health 10:519-524. Operation: Operation: Exposure Route, Aquatic (frishwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Paint Metric Taxa, Species, Age: Vertebrate; Fish; Pinephales promelas; Adult Metric Mortality Chemical: Distributes (DEHP) HERI DU: 791717 Domain Metric Rating Comments Comments Domain 1: Test Substance Metric 2: Test Substance Identity Metric 2: Test Substance Identity High The chemical was identified by name. Metric 3: Test Substance Purity Low The source was not reported. Domain 2: Test Design Metric 6: Negative Controls Low Metric 6: Radomized Allocation Low The source was not reported. Domain 3: Exposure Char-terization Metric 6: Randomized Allocation Low Metric 6: Radomized Allocation Low The isological response of the negative control. Metric 7: Reparation Low Researchers did not r	Study Citation:	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace							
Exposure Route, Aquatic (freshwader); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Pinephales promelas; Adult Media, Path: Taxa, Species, Age: Vertebrate; Fish; Pinephales promelas; Adult Metric Di-ethylhexyl phthalate (DEHP) IEKO ID: 79171 Domain Metric Metric 2: Test Substance Identity Metric 3: Test Substance Source Low The source was not reported. Metric 5: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Low Metric 7: Experimental System/Test Media Preparation Metric 8: Controls Randomized Allocation Metric 9: Reparation Metric 10: Experimental System/Test Media Preparation Metric 11: Substance Metric 12: Experimental System/Test Media Preparation Metric 8: Concentration Metric 11: Number of Exposure Metric 12: Experimental System/Test Media Preparation	Duration:	Substances i	n Environmental Health 10:519-524. ation: > 21 days: Exposure Duration: > 21	1 days					
Media, Path: Vertebrate; Fish; Pimephales promelas; Adult Taxa, Species, Age Vertebrate; Fish; Pimephales promelas; Adult Metall Outcome: Mortality Chemical: Di-ethylhexyl pithalate (DEHP) TERO ID: 717- Domain 1: Test Substance Metric 1: Test Substance Identity High Metric 2: Test Substance Identity Metric 3: Test Substance Source Low The source was not reported. Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Low Metric 7: Experimental System/Test Media Metric 6: Randomized Allocation Domain 3: Exposure Characterization High Metric 7: Experimental System/Test Media Preparation Scribestance Metric 1: Scribestance Metric 9: Metric 4: Negative Control Response High Domain 3: Exposure Characterization Scribestance Metric 7: Experimental System/Test Media	Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e., chemical of inter	est in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Vertebrate: Fish: Principales promelas; Adult Health Outcome: Mortality Chemical: Di-ethylhexyl phthalate (DEHP) Theath Outcome: Metric Rating Comments Domain Metric Rating Comments Domain 1: Test Substance Metric Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The unity/grade of the test substance was not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Regative Controls Low It was unclear if the author used a negative control group was no reported. Domain 3: Exposure Characterization Low The biological response of the negative control group was no reported. Metric 6: Randomized Allocation Low The success and not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Experimental System/Test Media Preparation High The experimental system and the methods for preparation of the test media were de- scribed in adequate deta	Media, Path:								
Intention Contentine Monitarily (Deminical) Monitarily (Defminical) Monitarily (Defminical) Chemicalis Di-ethylhexyl phthalate (DEHP) HERO ID: 791717 Domain 1: Test Substance Metric 1: Metric 2: Test Substance Identity Metric 2: High Test Substance Pointed Metric 3: Test Substance Pointed Domain 2: Test Design Metric 4: Negative Controls Metric 6: Metric 4: Negative Control Response Low Metric 6: Radomized Allocation Low The biological response of the negative control group was not reported. Metric 6: Radomized Allocation Domain 3: Exposure Characterization Metric 7: Exposure Characterization Metric 8: Metric 9: Mesurement of Test Substance Metric 10: Exposure Duration and Frequency Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Kirci 11: Metric 12: Testing at or Below Solubility Limit High The unber of exposure groups and the spacing of Exposure reversed consistently across study groups.	Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult Mortality						
HERO ID: 791717 France Comments Domain Metric Rating Comments Domain 1: Test Substance Metric 2: Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The purity/grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Metric 9: Measurement of Test Substance High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 10: Exposure Duration and Frequency High Researchers used a radio labelled chemical to monitor concentrations. Metric 11: Number of Exposure Groups/ High A flow-through	Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The source was not reported. Domain 2: Test Design Metric 5: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Metric 9: Metric 10: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 10: Metric 11: Number of Exposure Groups/ High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration Exposure Duration and Frequency	HERO ID:	791717							
Domain 1: Test Substance Metric 1: Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The purity/grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 9: Metric 9: Kasurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Consistently concentration Consistently consistently consolution and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Fayosure Duration and Frequency High A flow-through design with an appropriate duration wer	Domain		Metric	Rating	Comments				
Metric 1: Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The purity/grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 9: Metric 10: Exposure for preparation Metric 10: Exposure Duration and Frequency High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration High The unmber of exposure groups and the spacing of exposure levels were adequate. Metric 12:	Domain 1: Test Substan	ce		TT' 1					
Interfer 2: Test Substance Source Low The source was not reported. Metric 3: Test Substance Purity Low The purity/grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low It was unclear if the author used a negative or a solvent control. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 10: Exposure Duration and Frequency High Researchers used a radio labelled chemical to monitor concentrations. Concentration Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Metric 12: Test Organism Spacing of Exposure Levels High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.		Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
Internet 3. Test studistance runny Low The punity/grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 19: Metric 10: Exposure Duration and Frequency High Researchers used a radio labelled chemical to monitor concentrations. Concentration Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. <td></td> <td>Metric 2:</td> <td>Test Substance Source</td> <td>Low</td> <td>The source was not reported.</td>		Metric 2:	Test Substance Source	Low	The source was not reported.				
Domain 2: Test Design Metric 4: Negative Controls Low It was unclear if the author used a negative or a solvent control. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Metric 10: Exposure Duration and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Test organism High Exposure concentrations were at or below the water solubility limit and used acetone to aid		wienie 5.	Test Substance Funty	LOw	The purity/grade of the test substance were not reported.				
Metric 4:Negative ControlsLowIt was unclear if the author used a negative or a solvent control.Metric 5:Negative Control ResponseLowThe biological response of the negative control group was not reported.Metric 6:Randomized AllocationLowResearchers did not report how organisms were allocated to study groups.Domain 3: Exposure CharacterizationMetric 7:Experimental System/Test Media PreparationHighThe experimental system and the methods for preparation of the test media were de- scribed in adequate detail.Metric 8:Consistency of Exposure AdministrationHighDetails of the exposure administration were reported, and exposures were administered consistently across study groups.Metric 10:Exposure Duration and FrequencyHighResearchers used a radio labelled chemical to monitor concentrations. ConcentrationMetric 11:Number of Exposure Groups/HighA flow-through design with an appropriate duration were reported.Metric 12:Testing at or Below Solubility LimitHighExposure concentrations were at or below the water solubility limit and used acetone to aid solubility.Domain 4: Test OrganismTest OrganismHighExposure concentrations were at or below the water solubility limit and used acetone to aid solubility.	Domain 2: Test Design								
Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration Ketric 10: Exposure Groups/ High Metric 10: Exposure Groups/ High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Test Organism High Exposure concentrations were at or below the water solubility limit and used acetone to aid solu		Metric 4:	Negative Controls	Low	It was unclear if the author used a negative or a solvent control.				
Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation High The experimental system and the methods for preparation of the test media were described in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration Concentration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Metric 12: High A flow-through design with an appropriate duration were reported. Metric 12: Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Low Sacing of Exposure Low A		Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.				
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were de-scribed in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration High A flow-through design with an appropriate duration were reported. Metric 10: Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Netric 12: Test organism High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.		Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High The experimental system and the methods for preparation of the test media were de-scribed in adequate detail. Metric 8: Consistency of Exposure High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Concentration Concentration High Researchers used a radio labelled chemical to monitor concentrations. Metric 10: Exposure Duration and Frequency High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Domain 4: Test Organism Spacing of Exposure Levels Spacing of Exposure Levels	Damain 2. Ennance Ch								
Metric 7. Experimental System/Test Media Fign Fign Fign Fign Fign Fign Fign Fign Streperimental system and the methods for preparation of the test media were de- scribed in adequate detail. Metric 8: Consistency of Exposure Administration High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Metric 10: Exposure Duration and Frequency High Researchers used a radio labelled chemical to monitor concentrations. Metric 10: Exposure Duration and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Fign High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Exposure Fign High Exposure concentrations were at or below the water solubility limit and used acetone to	Domain 3: Exposure Ch	Matria 7	Experimental System/Test Media	Uich	The experimental system and the methods for monometics of the test mode were do				
Metric 8: Consistency of Exposure Administration High Details of the exposure administration were reported, and exposures were administered consistently across study groups. Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Metric 10: Exposure Duration and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Spacing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Test Organism Spacing of Exposure Levels Spacing of Exposure Levels		wieure 7.	Preparation	nigii	scribed in adequate detail.				
Metric 9: Measurement of Test Substance High Researchers used a radio labelled chemical to monitor concentrations. Metric 10: Exposure Duration and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Spacing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Limit Limit Limit Limit		Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.				
Metric 10: Exposure Duration and Frequency High A flow-through design with an appropriate duration were reported. Metric 11: Number of Exposure Groups/ High The number of exposure groups and the spacing of exposure levels were adequate. Spacing of Exposure Levels Spacing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Test Organism Test Organism		Metric 9:	Measurement of Test Substance	High	Researchers used a radio labelled chemical to monitor concentrations.				
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: High The number of exposure groups and the spacing of exposure levels were adequate. Metric 12: Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Test Organism Exposure concentrations were at or below the water solubility limit and used acetone to		Metric 10:	Exposure Duration and Frequency	High	A flow-through design with an appropriate duration were reported.				
Spacing of Exposure Levels Metric 12: Spacing of Exposure Levels Testing at or Below Solubility Limit High Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility. Domain 4: Test Organism Exposure concentrations were at or below the water solubility limit and used acetone to		Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.				
aid solubility. Domain 4: Test Organism		Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to				
Domain 4: Test Organism					aid solubility.				
	Domain 4: Test Organis	m							
Metric 13: Test Organism Characteristics Low The source (and sex if relevant) of the test animals was not reported.	8	Metric 13:	Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported.				
Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized.		Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
Conditions Matrice 15: Number of Organisms and Medium The numbers of test organisms and ranking (2) were reported and sufficient to share a		Matria 15	Conditions	Madium	The numbers of test arganisms and raplicates (2) wars reported and sufficient to shares				
Replicates per Group Replicates (5) were reported and sumclent to charac-		Methe 15.	Replicates per Group	Wiedium	terize toxicological effects.				
Domain 5: Outcome Assessment	Domain 5: Outcome Ass	sessment							
Metric 16: Adequacy of Test Conditions Medium Organism environmental conditions were conducive to the maintenance of health, though few details were provided.		Metric 16:	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.				
Metric 17: Outcome Assessment Methodology Low The outcome assessment methodology was not clearly reported.		Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.				
Metric 18: Consistency of Outcome Low Details of the outcome assessment protocol were not reported.		Metric 18:	Consistency of Outcome	Low	Details of the outcome assessment protocol were not reported.				
Continued on next page				Continued on next name					

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Mehrle, P. M	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace				
Duration:	Substances in Overall Dura	n Environmental Health 10:519-524. tion: > 21 days; Exposure Duration: > 21 da	iys			
Exposure Route,	Aquatic (fres	hwater); Water; Not determined by study auth	nors (i.e., chemical of inf	terest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	ish; <i>Pimephales promelas</i> ; Adult				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	791717					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Analy	vsis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Low	Data were only reported as a narrative.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Additional Comments.	NUILE					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace					
Duration:	Substances i Overall Dura	In Environmental Health 10:519-524. ation: > 21 days; Exposure Duration: > 21 d	lays			
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., chemical of in	nterest in exposure water, but unable to determine exact uptake route)		
Media, Path:	Verteberter Eiche Dimentation Adult					
Taxa, Species, Age: Health Outcome	Developmer	risn; <i>Pimephales prometas</i> ; Adult				
Chemical:	Di-ethylhex	yl phthalate (DEHP)				
HERO ID:	791717					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	nce	— — — — — — — — — —				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	It was unclear if the author used a negative or a solvent control.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure C	haracterization					
Domain 5. Exposure e	Metric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-		
		Preparation	6	scribed in adequate detail.		
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Researchers used radio labelled chemical to monitor concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	The study used a flow-through design with an appropriate duration.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The exposure concentrations were at or below the water solubility limit and used ace-		
				tone to aid solubility.		
Domain 4: Test Organi	sm					
C	Metric 13:	Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15.	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates (2) were reported and sufficient to shore a		
	Methe 15.	Replicates per Group	Medium	terize toxicological effects.		
		Replicates per Gloup				
Domain 5: Outcome A	ssessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome	Low	Details of the outcome assessment protocol were not reported.		
		Assessment				
		С	ontinued on next page			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Mehrle, P. M	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace					
Duration: Exposure Route,	Substances in Environmental Health 10:519-524. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	N (1 (D						
Taxa, Species, Age:	Vertebrate; F	ish; <i>Pimephales promelas</i> ; Adult					
Chemical:	Di-ethylbeyy	(Growin I phthalate (DEHP)					
HERO ID:	791717	791717					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	/ Variable Con	trol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Presenta	ation and Analy	vsis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	Low	Data were only reported as a narrative.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						

Overall Quality Determination

Uninformative

Study Citation:	Zanotelli, V	Zanotelli, V., Neuhauss, S., Ehrengruber, M. (2010). Long-term exposure to bis(2-ethylhexyl)phthalate (DEHP) inhibits growth of guppy fish (Poecilia					
Duration: Exposure Route, Media Path	reticulata). J Overall Dura Aquatic (fre	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate: I	Fish: <i>Poecilia reticulata</i> : Iuvenile					
Health Outcome	Developmen	of/Growth					
Chemical:	Di-ethylbex	Di-ethylhexyl nhthalate (DFHP)					
HERO ID:	697429	() philiadate (DEFIT)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	High	The DEHP was procured from Sigma.			
	Metric 3:	Test Substance Purity	High	Purity was reported as >98%, industrial grade.			
Domain 2: Test Design							
Ū.	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.			
	Metric 5:	Negative Control Response	High	The biological responses were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or the test media preparation methods were adequately reported.			
	Metric 8:	Consistency of Exposure	Medium	It wasn't clear if control solutions were renewed with the same schedule as the chemical treatment solutions.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	Minor limitations in exposure frequency and duration of exposure were identified. In- creased frequency of renewals may have been beneficial.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The high exposure concentration exceeded the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized or whether pretreat- ment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome Ats	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were ade- quate.			
Continued on next page							

May 2025

Environmental Hazard Evaluation

HERO ID: 697429 Table: 1 of 1

		contin	ued from previ	ous page		
Study Citation:	Zanotelli, V. reticulata), J	Zanotelli, V., Neuhauss, S., Ehrengruber, M. (2010). Long-term exposure to bis(2-ethylhexyl)phthalate (DEHP) inhibits growth of guppy fish (Poecilia reticulata). Journal of Applied Toxicology 30(1):29-33.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Poecilia reticulata; Juvenile					
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	697429					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	/ Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures	i iigii			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data Present	Motric 21:	Statistical Methods	High	Statistical methods were clearly decoribed		
	Metric 21.	Benerting of Date	Ligh	Deta for averaging related for lines were researted for each treatment and control group		
	Metrie 22.	Explanation of University of Outcomes	nigii Madiana	Data for exposure-related indings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in now the study characterized unex- pected outcomes.		
	None					

PUBLIC RELEASE DRAFT

Diethylhexyl Phthalate

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jee, J. H., K	Jee, J. H., Koo, J. G., Keum, Y. H., Park, K. H., Choi, S. H., Kang, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl-				
Duration: Exposure Route, Media, Path:	cholinesteras Overall Dura Aquatic (fres	se activity in bagrid catfish, Pseudobagrus ation: > 21 days; Exposure Duration: > 2 . shwater); Water, Food/Diet; Dietary	fulvidraco (R l days	ichardson). Journal of Applied Ichthyology 25(6):771-775.		
Taxa, Species, Age:	Vertebrate; F	Fish; Pseudobagrus fulvidraco; Adult				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1335887					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	The chemical grade was reported as analytical.		
Domain 2: Test Design						
e	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a		
	Matria 12	Spacing of Exposure Levels	NT/A	dose response.		
	Metric 12:	Testing at of Below Solubility Linit	IN/A	The exposure was via diet.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.		
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.		
	Metric 15:	Conditions Number of Organisms and	Low	The initial number of organisms was adequate, but replicates were not used.		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
Continued on next page						

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zard Evaluation

HERO ID: 1335887 Table: 1 of 3

		contin	ued from p	previous page			
Study Citation:	Jee, J. H., K cholinesteras	Jee, J. H., Koo, J. G., Keum, Y. H., Park, K. H., Choi, S. H., Kang, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl- cholinesterase activity in bagrid catfish, Pseudobagrus fulvidraco (Richardson). Journal of Applied Ichthyology 25(6):771-775.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water, Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Pseudobagrus fulvidraco; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1335887						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	Variable Co	atral					
Domain 0. Comountaing	Metric 10:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	Methe 19.	Design and Procedures	Ingn	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	veis					
Domain 7: Data Present	Metric 21:	Statistical Methods	High	Statistical methods were adequately described			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Jee, J. H., K	Coo, J. G., Keum, Y. H., Park, K. H., Chose activity in baorid catfish Pseudobaorus	oi, S. H., Ka fulvidraco (R	ng, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl- ichardson). Journal of Applied Johthyology 25(6):771-775
Duration: Exposure Route	Overall Dura	ation: > 21 days; Exposure Duration: > 21	l days	ionardson). Joanna or reprice toningology 20(0). 771 775.
Media. Path:	Aquatic (free	silwater), water, Pood/Diet, Dietary		
Taxa, Species, Age:	Vertebrate; F	Fish; <i>Pseudobagrus fulvidraco</i> ; Adult		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	1335887			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical grade was reported as analytical.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-
	metho /.	Preparation	mgn	scribed in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a
		Spacing of Exposure Levels	ing.	dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organis	m			
C	Metric 13:	Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The initial number of organisms was adequate, but replicates were not used.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

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Environmental Hazard Evaluation

HERO ID: 1335887 Table: 2 of 3

continued from previous page						
Study Citation:	Jee, J. H., K	oo, J. G., Keum, Y. H., Park, K. H., Cho	oi, S. H., Ka	ng, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl-		
Duration	cholinesteras	cholinesterase activity in bagrid catfish, Pseudobagrus fulvidraco (Richardson). Journal of Applied Ichthyology 25(6):771-775.				
Euroguna Douto	A quatia (frag	where the product of	uays			
Exposure Koule,	Aquatic (free	silwater); water, Food/Diet; Dietary				
Media, Path:	X 7 , 1 , T					
Taxa, Species, Age:	Vertebrate; F	ish; <i>Pseudobagrus fulvidraco</i> ; Adult				
Health Outcome:	Mortality	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1335887					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Coi	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Procont	tation and Anal	voic				
Domain 7. Data Fiesen	Metric 21.	ysis Statistical Methods	Low	Statictical analysis may have been performed but not described adequately		
	Metric 22:	Reporting of Data	High	Data for exposure related findings were presented for each treatment and control group		
	Matria 22.	Explanation of Unexpected Outcomes	Ligh	There were no unsurgested outcomes		
	Wieute 25.	Explanation of Onexpected Outcomes	nigii	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quali	ty Detern	nination	High			

Study Citation:	Jee, J. H., K cholinesteras	Koo, J. G., Keum, Y. H., Park, K. H., Ch se activity in bagrid catfish. Pseudobagrus	oi, S. H., Ka fulvidraco (R	ng, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl- cichardson). Journal of Applied Ichthyology 25(6):771-775.
Duration: Exposure Route,	Overall Dura Aquatic (free	ation: > 21 days; Exposure Duration: > 2 shwater); Water, Food/Diet; Dietary	1 days	
Media, Path:	N / () (F			
Taxa, Species, Age:	Vertebrate; F	Pish; <i>Pseudobagrus fulvidraco</i> ; Adult		
Chemical.	Di-ethylbexy	<i>I</i> phthalate (DFHP)		
HERO ID:	1335887			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical grade was reported as analytical.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	dose response. The exposure was via diet
	110010 12.	Testing at of Below Solutionty Limit	10/1	
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of organisms was adequate, but replicates were not used.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 1335887 Table: 3 of 3

		contir	nued from p	previous page		
Study Citation:	Jee, J. H., K cholinesteras	Jee, J. H., Koo, J. G., Keum, Y. H., Park, K. H., Choi, S. H., Kang, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl- cholinesterase activity in bagrid catfish, Pseudobagrus fulvidraco (Richardson). Journal of Applied Ichthyology 25(6):771-775.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water, Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Pseudobagrus fulvidraco; Adult				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1335887					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion is for AChE activity.				
Overall Qualit	ty Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.						
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (fre	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Pungitius pungitius</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported ADME (biotransformation) Di-ethylhexyl phthalate (DEHP) 59542						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F ADME (bio Di-ethylhexy 59542							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure was reported.				
	Metric 2: Metric 3:	Test Substance Source	High	The source was identified as New England Nuclear.				
	Metric 5.	Test Substance Fullty	nigii	The purity was 99.5%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.				
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.				
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.				
Domain 3: Exposure Ch	aracterization							
2 children et 2.4postate et	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and the placement of organisms in 20L tanks were not reported.				
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system.				
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).				
	Metric 10:	Exposure Duration and Frequency	High	A 27-day exposure was sufficient.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one exposure concentration was utilized in this study.				
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than approximately solubil- ity in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age or sex was not provided in the study.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	No replicates were reported.				
Domain 5: Outcome As	sessment	Replicates per Group						

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page		
Study Citation:	Sodergren, A Environmen	A. (1982). Significance of interfaces in the distr tal Pollution 27(4):263-274.	ibution and metaboli	sm of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.		
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 day	ys			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Pungitius pungitius; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	59542					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of feed for fish were not well described.		
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).		
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms were loaded into the same experimental tank. This could affect 14C DEHP uptake by each organism.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal at- trition or health outcomes unrelated to the exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.		
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.		
Additional Comments:	This study of mesocosm. (may not be a DEHP + met	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites the metabolites being phthalic acid and phthalic anhydride.				

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	IVL, (2001).	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,							
Duration: Exposure Route, Media. Path:	Rana arvalis Overall Dura Aquatic (fres	Rana arvalis. Dverall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana arvalis; Embryo							
Health Outcome:	Developmen	t/Growth							
Chemical:	Di-ethylhexy	l phthalate (DEHP)							
HERO ID:	7328184								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.					
	Metric 2:	Test Substance Source	High	The DEHP was donated from Neste Oxo and was verified by gas chromatography.					
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.9%.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control and a solvent control with acetone.					
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Tables 10 and 11 and in Figure 6 and were adequate for the outcomes of interest.					
	Metric 6:	Randomized Allocation	Medium	Frog eggs were reported to be randomly distributed into study groups.					
	, . . .								
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The tests were reported to occur in 3L glass beakers with 400g of soil dosed with the test substance and 2L of synthetic lake water. The soil was prepared by dissolving the					
				phthalate in acetone and mixing it in uncontaminated homogenized, air-dried sediment. The solvent was then evaporated and the soil was added to more uncontaminated soil to achieve the proper concentration.					
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 35d in 3L glass beakers with 400g sediment and 2L of artificial lake water. They were all aerated for the duration of the study. All test chambers were covered.					
	Metric 9:	Measurement of Test Substance	High	The test substance was reported to be measured using GC analysis.					
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for the tests at 5C were reported to last for 35d and was adequate for the outcomes of interest.					
	Metric 11:	Number of Exposure Groups/	Medium	There were 3 reported exposure groups, which is less than is typical, but this was ade- quate for the outcomes of interest. Spacing was adequate as well					
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.					
Domain 4: Test Organisi	n								
	Metric 13:	Test Organism Characteristics	Low	The test organisms were collected from a small island in Umealvens in Umea Sweden. This creates concerns about animal health.					
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the eggs were acclimated prior to the start of the test.					
		Cont	inued on nex	t page					

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Environmental Hazard Evaluation

HERO ID: 7328184 Table: 1 of 4

		conti	nued from p	revious page		
Study Citation:	IVL, (2001).	. Further investigations on the influence of	sediment-ass	sociated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog		
Dunations	Rana arvalis					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	snwater); Sediment; Not determined by stu	dy authors (1	.e., chemical of interest in exposure water, but unable to determine exact uptake route		
Media, Path:	Mantalanataa	Analihian Dava analia Eashara				
Iaxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Chamicala	Diverte Direction Diversion (DEHP)					
	7328184	(DEHF)				
	7520104	N6 / *				
Domain	N 17	Metric	Rating	Comments		
	Metric 15:	Replicates per Group	Medium	There were approximately 50 eggs per test chamber with 5 replicates per treatment and control.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 5C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-time to embryo hatch, deformation rate, and growth.		
	Metric 18:	Consistency of Outcome Assessment	High	Hatch was monitored on days 22, 25, and 35 for the 5C studies, and tadpole develop- ment was assessed at the end of the study.		
Domain 6: Confounding	/ Variable Co	ntrol				
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Wieule 19.	Design and Procedures	Low	conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presenta	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section.		
	Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 10 and 11 and in Figure 6 and were adequate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study authors reported that some of the test sub- stance started to leach into the test water and could potentially affect the outcomes.		
Additional Comments:	This portion said embryo	of the study was on the effect of DEHP on s at 5C. The outcome selected was develop	hatch time, t ment/growth	adpole deformities, and tadpole weights in embryos and the tadpoles that hatched fror .		
Overall Qualit	v Dotorr	nination	High			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	IVL, (2001).	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,						
Duration:	Rana arvalis Overall Dura	ation: > 21 days; Exposure Duration: > 2	1 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taya Species Age:	Vertebrate: /	Vertebrate: Amphibian: Rana avalis: Embryo						
Health Outcome:	Mortality	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	7328184							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.				
	Metric 2:	Test Substance Source	High	The DEHP was donated from Neste Oxo and was verified by gas chromatography.				
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.9%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control and a solvent control with acetone.				
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Tables 10 and 11 and were adequate for the outcomes of interest.				
	Metric 6:	Randomized Allocation	Medium	Frog eggs were reported to be randomly distributed into study groups.				
Domain 3: Exposure Ch	aracterization		TT' 1					
	Metric 7:	Experimental System/Test Media Preparation	Hign	The tests were reported to occur in 3L glass beakers with 400g of soil dosed with the test substance and 2L of synthetic lake water. The soil was prepared by dissolving the phthalate in acetone and mixing it in uncontaminated homogenized, air-dried sediment. The solvent was then evaporated and the soil was added to more uncontaminated soil to achieve the proper concentration.				
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 35d in 3L glass beakers with 400g sediment and 2L of artificial lake water. They were all aerated for the duration of the study. All test chambers were covered.				
	Metric 9:	Measurement of Test Substance	High	The test substance was reported to be measured using GC analysis.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the tests at 5C were reported to last for 35d and was adequate for the outcomes of interest.				
	Metric 11:	Number of Exposure Groups/	Medium	There were 3 reported exposure groups, which is less than is typical, but was adequate				
	N . · · · · ·	Spacing of Exposure Levels	3.7/1	for the outcomes of interest.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.				
Domain 4. Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The test organisms were collected from a small island in Umealvens in Umea Sweden. This creates concerns about animal health.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the eggs were acclimated prior to the start of the test.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were approximately 50 eggs per test chamber with 5 replicates per treatment and				
		Replicates per Group						
Continued on next page								

HERO ID: 7328184 Table: 2 of 4

		conti	nued from p	revious page		
Study Citation:	IVL, (2001)	. Further investigations on the influence of	sediment-as	sociated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,		
Duration	Rana arvalis	$\frac{1}{2}$	l dave			
Exposure Route, Media Path	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa. Species. Age:	Vertebrate; Amphibian; Rana arvalis; Embryo					
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	7328184					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 5C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–egg and tadpole mortality.		
	Metric 18:	Consistency of Outcome Assessment	High	Hatch was monitored on days 22, 25, and 35 for the 5C studies, and tadpole survival was assessed at the end of the study.		
Domain 6: Confounding	v / Variabla Ca	ntrol				
Domain 0. Comounding	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical analyses were described in the "Statistical methods" section.		
	Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 10 and 11 and were ade- quate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study author reported that some of the test sub- stance started to leach into the test water and could potentially affect the outcomes.		
Additional Comments:	This portion was mortalit	of the study was on the effect of DEHP on y.	mortality in	embryos and the tadpoles that hatched from said embryos at 5C. The outcome selected		
Overall Quali	ty Deterr	nination	High			

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Study Citation:	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,						
Duration: Exposure Route, Modia, Path:	Rana arvalis Overall Dura Aquatic (free	Rana arvalis. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Di-ethylhexy 7328184	Amphibian; <i>Rana arvalis</i> ; Embryo yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	High High High	The DEHP was identified by CASRN. The DEHP was donated from Neste Oxo and was verified by gas chromatography. The purity was reported to be 99.9%.			
Damain 2. Taat Daaian							
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control and a solvent control with acetone.			
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Tables 8 and 9 and were adequate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Medium	Frog eggs were reported to be randomly distributed into study groups.			
	, . , .						
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	High	The tests were reported to occur in 3L glass beakers with 400g of soil dosed with the test substance and 2L of synthetic lake water. The soil was prepared by dissolving the phthalate in acetone and mixing it in uncontaminated homogenized, air-dried sediment. The solvent was then evaporated and the soil was added to more uncontaminated soil to achieve the proper concentration.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 26d in 3L glass beakers with 400g sediment and 2L of artificial lake water. They were all aerated for the duration of the study. All test chambers were covered.			
	Metric 9:	Measurement of Test Substance	High	The test substance was reported to be measured using GC analysis.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the tests at 10C was reported to last for 26d and was adequate for the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 3 reported exposure groups, which is less than is typical, but was adequate for the outcomes of interest.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.			
Domain 4: Test Organis	m Motric 12:	Test Organism Characteristics	Law	The test executions were calledted from a small intend in the share in these 0			
	ivietric 13:	Test Organism Characteristics	Low	The test organisms were collected from a small island in Umealvens in Umea Sweden. This creates concerns about animal health.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the eggs were acclimated prior to the start of the test.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were approximately 50 eggs per test chamber with 5 replicates per treatment and control.			
			•1				

HERO ID: 7328184 Table: 3 of 4

			nueu nom p				
Study Citation:	IVL, (2001)	. Further investigations on the influence of	sediment-as	sociated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,			
- -	Rana arvalis	Rana arvalis.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days A quatic (freehyster). Sodiment. Not determined by study outbors (i.e., shemical of interact in averaging water but weekle to determine exact wreters route).					
Exposure Route,	Aquatic (fre	shwater); Sediment; Not determined by stu	idy authors (1	.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	T 7 . 1 .						
Taxa, Species, Age:	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	/328184						
Domain		Metric	Rating	Comments			
Domain 5. Outcome As	accoment						
Domain 5: Outcome As	Matria 16	Adequacy of Test Conditions	Madium	The test was conducted at 10C with a 121 12D photoporied in supporting lake water. The			
	Metric 10.	Adequacy of rest Conditions	Medium	water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–egg and tadpole mortality.			
	Metric 18:	Consistency of Outcome	High	Hatch was monitored on days 9, 12, 16, and 21 for the 10C studies, and tadpole survival			
		Assessment		was assessed at the end of the study.			
Domain 6: Confounding	. / Variable Co	ntrol					
Domain 0. Comounding	Metric 10.	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	Mettie 17.	Design and Procedures	Low	conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7. Data Present	ation and Anal	lycic					
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section			
	Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 8 and 9 and were adequate			
	Weute 22.	Reporting of Data	Ingn	for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study authors reported that some of the test sub- stance started to leach into the test water and could potentially affect the outcomes.			
Additional Comments:	ts: This portion of the study was on the effect of DEHP on mortality in embryos and the tadpoles that hatched from said embryos at 10C. The outcome selected was mortality.						
Overall Qualit	ty Deterr	nination	High				

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Study Citation:	IVL, (2001)	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,					
Duration: Exposure Route, Media, Path:	Rana arvalis Overall Dur Aquatic (fre	Rana arvalis. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; A Developmer Di-ethylhexy	Amphibian; <i>Rana arvalis</i> ; Embryo ht/Growth yl phthalate (DEHP)					
HERO ID:	/328184						
Domain		Metric	Rating	Comments			
Domain 1: Test Substa	nce Matria 1.	Test Substance Identity	High				
	Metric 1: Matria 2:	Test Substance Identity	High	The DEHP was identified by CASKN.			
	Metric 2: Matria 3:	Test Substance Source	High	The purity was consided from Neste Oxo and was verified by gas chromatography.			
	Metric 5.	Test Substance Purity	пign	The purity was reported to be 99.9%.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control and a solvent control with acetone.			
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Tables 8 and 9 and in Figure 5 and were adequate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Medium	Frog eggs were reported to be randomly distributed into study groups.			
Domain 3: Exposure C	haracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The tests were reported to occur in 3L glass beakers with 400g of soil dosed with the test substance and 2L of synthetic lake water. The soil was prepared by dissolving the phthalate in acetone and mixing it in uncontaminated homogenized, air-dried sediment. The solvent was then evaporated and the soil was added to more uncontaminated soil to achieve the proper concentration.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 26d in 3L glass beakers with 400g sediment and 2L of artificial lake water. They were all aerated for the duration of the study. All test chambers were covered.			
	Metric 9:	Measurement of Test Substance	High	The test substance was reported to be measured using GC analysis.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the tests at 10C were reported to last for 26d and was ade- quate for the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 3 reported exposure groups, which is less than is typical but was adequate for the outcomes of interest. Spacing was adequate as well.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.			
Domain 1. Test Organi	em						
Domain 4. Test Organi	Metric 13:	Test Organism Characteristics	Low	The test organisms were collected from a small island in Umealvens in Umea Sweden. This creates concerns about animal health.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the eggs were acclimated prior to the start of the test.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were approximately 50 eggs per test chamber with 5 replicates per treatment and			
		Replicates per Group		control.			

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Environmental Hazard Evaluation

HERO ID: 7328184 Table: 4 of 4

		conti	nued from p	revious page		
Study Citation:	IVL, (2001).	. Further investigations on the influence of	sediment-ass	sociated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog,		
Duration:	Rana arvalis	ation: 11 - 21 days: Exposure Duration: > 3	21 days			
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; Amphibian; Rana arvalis; Embryo					
Health Outcome:	Development/Growth					
Chemical: HERO ID:	Di-ethylhexy 7328184	yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 10C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-time to embryo hatch, deformation rate, and growth.		
	Metric 18:	Consistency of Outcome Assessment	High	Hatch was monitored on days 9, 12, 16, and 21 for the 10C studies and tadpole develop- ment was assessed at the end of the study.		
Domain 6: Confounding	y / Variable Co	ntrol				
Domain 0. Comounding	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section.		
	Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 8 and 9 and in Figure 5 and were adequate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study author reported that some of the test sub- stance started to leach into the test water and could potentially affect the outcomes.		
Additional Comments:	This portion said embryo	of the study was on the effect of DEHP on s at 10C. The outcome selected was develo	hatch time, t pment/growt	adpole deformities, and tadpole weights in embryos and the tadpoles that hatched from h.		
Overall Oualit	ty Detern	nination	High			

Study Citation:	IVL, (1997).	The influence of sediment-associated phtha	alate esters (DEHP and DID	DP) on hatching and survival of the moorfrog, Rana arvalis.
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days	
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Vertebrate; A	Amphibian; <i>Rana arvalis</i> ; Embryo		
Health Outcome:	Behavioral			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	7978546			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.
Domain 2: Test Design				
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Uninformative	The biological response of the control was not reported for motility.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Cr	naracterization			
	Metric /:	Experimental System/ Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the
	Matria 8.	Preparation Consistency of Exposure	Madium	The study provided for details on the experimentation
	Metric o.	A dministration	Medium	The study provided few defails on the exposure administration.
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations varied considerably.
	Metric 10:	Exposure Duration and Frequency	Low	The duration was appropriate, but the lack of renewals led to fungal infections.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organis	sm			
	Metric 13:	Test Organism Characteristics	Low	The test organisms were wild caught. It is unclear at what development stage the expo- sure was initiated.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 raplicates for 50 total
	Weute 15.	Replicates per Group	Wiedrum	There were to organisms with 5 replicates for 50 total.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
		(Continued on next page	

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 1 of 16

		C	ontinued from previous p	bage				
Study Citation:	IVL, (1997).	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana arvalis; Embryo						
Health Outcome:	Behavioral	Behavioral						
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	7978546							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were con-				
	Assessment fusing.							
Domain 6: Confounding	g / Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Uninformative	No statistics were conducted for motility.				
	Metric 22:	Reporting of Data	Uninformative	No quantitative data was presented on motility. No dose-specific qualitative data was presented either.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				
Additional Comments:	None							
Overall Ouali	Overall Ouality Determination Uninformative							
Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
---	---	---	---------	---	--			
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; A ADME (biot Di-ethylbey)	Amphibian; <i>Rana arvalis</i> ; Embryo ransformation) d phthalate (DEHP)						
HERO ID:	7978546							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.				
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was reported and reasonable, but there was some contamination in the control water.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the				
	Wieure 7.	Preparation	Wiedium	experimental system was not described adequately				
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.				
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations varied considerably.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.				
				*				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.				
		Replicates per Group						
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were con- fusing.				

Continued on next page ...

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 2 of 16

continued from previous page						
Study Citation:	IVL, (1997)	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical for this outcome.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad- pole.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quality Determination Low						

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Study Citation: Duration: Exposure Route, Media Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome:	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo Mortality					
Chemical: HERO ID:	Di-ethylhexy 7978546	Di-ethylhexyl phthalate (DEHP) 7978546				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.		
Domain 2: Test Design						
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.		
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.		
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.		
Domain 4: Test Organis	m					
U	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
Continued on next page						

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 3 of 16

continued from previous page						
Study Citation:	IVL, (1997)	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (fre	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; <i>Rana arvalis</i> ; Embryo				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	7978546	7978546				
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	tation and Anal	veis				
Domain 7. Data 1 10501	Metric 21.	Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal		
	Metric 21.	Statistical Wethous	LOW	of some samples due to infection was somewhat arbitrary.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad- pole.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This evaluat	ion is for survival.				
Overall Quality Determination Low						

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana arvalis; Embryo						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP) 7978546						
	7976340							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	Notria 1.	Test Substance Identity	Low					
	Metric 1:	Test Substance Identity	LOW	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.				
	Metric 5:	Test Substance Purity	High	The chemical purity was reported as 99.0%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 2: Exposure Ch	araatarization							
Domain 5. Exposure Ci	Metric 7	Experimental System/Test Media	Madium	The methods for propagation of the tast modio wars described in adequate datail, but the				
	Wieurie 7.	Preparation	Wiedium	experimental system was not described.				
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.				
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations varied considerably.				
	Metric 10:	Concentration Exposure Duration and Erequency	Low	The duration was appropriate but look of renewals lod to fungel infections				
	Metric 11:	Number of Exposure Groups/	Low High	The number of exposure groups and the specing of exposure levels were suitable				
	Methe 11.	Spacing of Exposure Levels	Ingn	The number of exposure groups and the spacing of exposure levels were suitable.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.				
		Replicates per Group						
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome	Medium	The method and the timing of determining hatch were not clear.				

Domain 6: Confounding / Variable Control

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Amphibian; Rana arvalis; Embryo **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 7978546 Rating Domain Metric Comments Confounding Variables in Test Metric 19: Low Temperature variations were evident. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Low Fungal infections may have skewed the results. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control tadpole. Metric 23: Explanation of Unexpected Outcomes Low The study did not report any measures of variability. Additional Comments: This form was for survival and hatch. Medium **Overall Quality Determination**

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; A Developmen Di-ethylhex 7978546	Amphibian; <i>Rana arvalis</i> ; Embryo t/Growth yl phthalate (DEHP)			
Domain	1910540	Metric	Rating	Comments	
Domain 1: Test Substan	ce		Tuning		
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2. Test Design					
Domani 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
Domain 5. Exposure er	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not described adequately	
	Metric 8.	Consistency of Exposure	Medium	The study provided few details on exposure administration	
	Wieule 0.	Administration	Wiedium	The study provided few details on exposure administration.	
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations varied considerably.	
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m				
2 olimani il 1000 oliganio	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		ited.	
		Conti	nued on next pa	ge	

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days **Exposure Route**, Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Amphibian; Rana arvalis; Embryo **Health Outcome:** Development/Growth Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 7978546 Domain Metric Rating Comments Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low Temperature variations were evident. **Design and Procedures** Metric 20: Outcomes Unrelated to Exposure Low Fungal infections may have skewed the results. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control tadpole. Metric 23: Explanation of Unexpected Outcomes Low The study did not report any measures of variability. Additional Comments: This evaluation was for weight and % lipids.

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A ADME (biot Di-ethylhexy 7978546	Amphibian; <i>Rana arvalis</i> ; Embryo transformation) yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of test media were described in adequate detail, but the experimental system was not adequately described.	
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.	
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.	
	Metric 10:	Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		Ited.	
Continued on next page					

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 6 of 16

continued from previous page						
Study Citation:	IVL, (1997).	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical for this outcome.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quality Determination Low						

Duration: Exposure Route, Media Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A ADME (bior Di-ethylhexy 7978546	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo ADME (biotransformation) Di-ethylhexyl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice		8			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization					
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.		
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.		
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.		
	Metric 10:	Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.		
Domain 4: Test Organis	sm					
U	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.		
		Replicates per Group				
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 7 of 16

continued from previous page						
Study Citation:	IVL, (1997).	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical for this outcome.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quality Determination Low						

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo ADME (biotransformation) Di-ethylhexyl phthalate (DEHP) 7978546				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ice				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	naracterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.	
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.	
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m				
C	Metric 13:	Test Organism Characteristics	Low	The test organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		ited.	
Continued on next page					

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 8 of 16

continued from previous page						
Study Citation:	IVL, (1997).	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical this outcome.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quality Determination Low						

Study Citation: Duration: Exposure Route, Media Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Developmen Di-ethylhexy 7978546	Amphibian; <i>Rana arvalis</i> ; Embryo t/Growth yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ice				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	naracterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.	
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.	
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m				
2	Metric 13:	Test Organism Characteristics	Low	The test organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.	
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Diethylhexyl Phthalate

HERO ID: 7978546 Table: 9 of 16

continued from previous page						
Study Citation:	IVL, (1997).	VL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i	.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Con	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, n=1.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This evaluati	ion was for weight and % lipids.				
Overall Quality Determination Low						

Taxa, Species, Age: Vertebrate; Auphibian; Rana arvalis; Embryo Vertebrate; Auphibian; Rana arvalis; Embryo Health Outcome: Development/Growth Development/Growth Chemical: Text/Hucky/Fhhilalate (DEHP) HERO ID: 797854 Domain 1: Test Substance Metric 1: Test Substance Identity Low The chemical was identified by name only. Metric 2: Test Substance Source High DEHP was analytically verified using GC-MS. Metric 3: Test Substance Purity High The chemical purity was reported as 99.6%. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Metric 6: Randomized Allocation Low The subors reported using an appropriate concurrent negative control group. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Low The methods for preparation of the test media were described in adequate detail, but the caperation of the test media were described in adequate detail, but the caperation of the test media were described in adequate detail, but the caperation of the test media were described in adequate detail, but the caperation of the test media were described in adequate detail, but the caperation of the test media were described in adequate detail, but the concentration was appropriate, but tack of renewals le	Study Citation: Duration: Exposure Route, Media. Path:	IVL, (1997). Overall Dura Aquatic (free	The influence of sediment-associated pht ation: > 21 days; Exposure Duration: > 2 shwater); Sediment; Not determined by stu	thalate esters (1 days udy authors (i	(DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. .e., chemical of interest in exposure water, but unable to determine exact uptake route)
Health Outcome: Development/Growth Chemical: Di-teivth[hask] thithalate (DEHP) HERO ID: 7978546 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low The chemical was identified by name only. Metric 2: Test Substance Source High DEHP was analytically verified using GC-MS. Metric 3: Test Substance Purity High Detherical purity was reported as 99.6%. Domain 2: Test Design Metric 6: Regative Controls High Metric 5: Negative Control Response Low The behological response of the negative control group was not reported. Domain 3: Exposure Characterization Low The methods for preparation of the test media ware described in adequate detail, but the experimental system was not adequately described. Metric 7: Experimental System/Test Media Preparation Medium The methods for preparation of the test media ware described in adequate detail, but the exposure for the or consistence of Exposure Metric 10: Exposure Corrups/ Medium Water concentrations were measured, but concentrations deviated from nominal. Metric 11: Number of Exposure Corrups/ N/A Only one concentration wa	Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo		
Chemical: Di-ethymesyl pinnalact (DEFIP) 7978546 7978546 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low The chemical was identified by name only. Metric 2: Test Substance Identity Low The chemical purity was reported using GC-MS. Metric 3: Test Substance Purity High DEHP was analytically verified using GC-MS. Domain 2: Test Design Metric 6: Negative Controls High The chemical purity was reported using an appropriate concurrent negative control group. Metric 6: Randomized Allocation Low The biological response of the negative control group was not reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 8: Consistency of Exposure Medium The methods for preparation of the test media networe described in adequate detail, but the experimental system was not adequately described. Metric 10: Experimental System/Test Media Concentration Wetric 1: Naministration. Metric 11: Number of Test Substanc	Health Outcome:	Developmen	t/Growth		
Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low The chemical was identified by name only. Metric 2: Test Substance Source High DEHP was analytically verified using GC-MS. Metric 3: Test Substance Purity High The chemical purity was reported as 99.6%. Domain 2: Test Design Metric 6: Randomized Controls High Metric 5: Negative Control Response Low The biological response of the negative control group. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Preparation Metric 7: Experimental System/Test Media Medium The enchods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 9: Consistency of Exposure Medium The study provided few details on exposure administration. Metric 10: Exposure Duration and Frequency Low The duration was appropriate, but lack of renewals led to fungal infections. Metric 11: Number of Exposure Levels Medium The duration was appropriate, but lack of renewals led to fungal infe	HERO ID:	7978546	/I phthalate (DEHP)		
Domain 1: Test Substance Metric 1: Metric 2: Test Substance Source The chemical was identified by name only. DiffP was analytically verified using GC-MS. Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Regative Control Response High Low Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was not reported. Metric 6: Readomized Allocation Domain 3: Exposure Characterization Metric 8: Consistency of Exposure Medium Preparation Metric 9: Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Duration and Frequency Metric 12: Test Organism Medium Metric 12: Test Organism The was appropriate on exposure and infections. Metric 13: Metric 13: Test Organism Characteristics Domain 4: Test Organism Metric 14: Acclimatization and Pretreatment Conditions The test organisms were wild caught. It was unclear at what development stage the exposure was via sediment. Domain 4: Test Organism Metric 14: Number of Crganisms and Metric 15: Number of Organisms and Metric 15: Number of Organisms and Metric 15: Number of Organisms and Metric 15: Number of Organisms and Metric 16: Number of Organisms and Metr	Domain		Metric	Rating	Comments
Metric 1:Test Substance Identity Metric 2:Low Test Substance Source High DEHP was analytically verified using GC-MS. DEHP was analytically verified using GC-MS. High The chemical purity was reported as 90.6%.Domain 2: Test Design Metric 3:Metric 4:Negative Controls Negative Control Response Metric 6:High High Nuthow Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was not reported. Metric 6:Domain 3: Exposure Characterization Metric 7:Experimental System/Test Media Preparation Consistency of ExposureMedium Medium Medium The methods for preparation of the test media were described in adequate detail, but the experimental system/Test Substance Consistency of ExposureMedium Medium Medium The study provided few details on exposure administration. Administration Metric 19:Metric 10:Exposure Duration and Frequency Spacing of Exposure Levels Metric 11:Medium Spacing of Exposure Levels N/ADomain 4: Test Organism Metric 13:Test Organism CharacteristicsLow LowThe exposure was via sediment.Domain 4: Test Organism Metric 14:Acclimatization and Prequency Spacing of Exposure Levels N/AN/AThe exposure was via sediment.Domain 4: Test Organism Metric 15:Test Organism CharacteristicsLow LowThe study did not report whether test organisms were acclimatized.Domain 4: Test Organism Metric 15:Test Organism and Preparation and Pretreatment Conditions Number of Organisms and Number of Organisms and Number of Organisms and Number of Organisms and Number of O	Domain 1: Test Substand	ce			
Metric 2: Metric 3:Test Substance Source Test Substance PurityHigh HighDEHP was analytically verified using GC-MS. High The chemical purity was reported as 99.6%.Domain 2:Test Design Metric 4:Negative Controls Negative Control Response Metric 6:High Ruber Substance PurityStudy authors reported using an appropriate concurrent negative control group. Low The biological response of the negative control group was not reported. LowDomain 3:Exposure Characterization Metric 6:Experimental System/Test Media Preparation (Consistency of Exposure MediumMedium Metium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Medium Metric 9:Medium Medium Medium MediumThe unation of the test media were described in adequate detail, but the experimental system was not adequately described. MediumMetric 9:Measurement of Test Substance Measurement of Test SubstanceMedium Nater concentrations were measured, but concentrations deviated from nominal. Concentration Exposure Duration and Frequencey Spacing of Exposure Levels Metric 12:NANater concentration was appropriate, but lack of renewals led to fungal infections.Domain 4:Test Organism Metric 13:Test Organism CharacteristicsLowThe exposure was via sediment.Domain 4:Test Organism Metric 14:Acclimatization and Pretreatment ConditionsLowThe study did not report whether test organisms were acclimatized. ConditionsDomain 4:Test Organism Number of Organisms and Netric 15:Nater Preparation Medium		Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
Metric 3:Test Substance PurityHighThe chemical purity was reported as 99.6%.Domain 2: Test DesignMetric 4:Negative ControlsHighStudy authors reported using an appropriate concurrent negative control group. LowMetric 5:Negative Control ResponseLowThe biological response of the negative control group was not reported. LowDomain 3: Exposure CharacterizationMetric 7:Experimental System/Test Media PreparationMediumMetric 8:Consistency of ExposureMediumThe methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.Metric 9:Metric 9:MediumThe study provided few details on exposure administration.Metric 10:Exposure Duration and Frequency Spacing of Exposure Groups/ Spacing of Exposure Levels Metric 11:N/AOnly one concentration was used.Domain 4: Test OrganismMetric 13:Test Organism CharacteristicsLowThe test organisms were wild caught. It was unclear at what development stage the exposure was initiated.Domain 4: Test OrganismMetric 14:Acclimatization and Preteatment Conditions Metric 15:LowThe test organisms were wild caught. It was unclear at what development stage the exposure was initiated.Domain 4: Test OrganismMetric 16:Test Organisms and Netric 15:MediumThe rewere 10 organisms with 5 replicates for 50 total.		Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.
Domain 2: Test Design Metric 4: Negative Controls High Low Study authors reported using an appropriate concurrent negative control group. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 8: Consistnery of Exposure Medium The study provided few details on exposure administration. Metric 9: Measurement of Test Substance Medium Weare concentrations were measured, but concentrations deviated from nominal. Concentration Concentration and Frequency N/A Only one concentration was appropriate, but lack of renewals led to fungal infections. Metric 12: Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Test Organisms and Pretreatment Low The tstudy did not report whether test organisms were acclimatized. Metric 13: Number of Organisms and Prevention N/A The evere 10 organisms with 5 replicates for 50 total.		Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.
Metric 4: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Metric 5: Negative Control Response Low The biological response of the negative control group was not reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 8: Consistency of Exposure Medium The study provided few details on exposure administration. Metric 10: Exposure Duration and Frequency Metric 11: Number of Test Substance Medium Water concentration was appropriate, but lack of renewals led to fungal infections. Metric 12: Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Test Organism Characteristics Low The test organisms were avaited. Metric 13: Test Organism Characteristics Low The test organisms were advalued. Metric 14: Acclimatization and Pretreatment Conditions Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Domain 4: Test Organism Metric 13: Test Organism characteristics Low <t< td=""><td>Domain 2: Test Design</td><td></td><td></td><td></td><td></td></t<>	Domain 2: Test Design				
Metric 5: Metric 6: Negative Control Response Randomized Allocation Low Low The biological response of the negative control group was not reported. Low The biological response of the negative control group was not reported. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 8: Consistency of Exposure Administration Medium The study provided few details on exposure administration. Metric 9: Measurement of Test Substance Concentration Medium Water concentrations were measured, but concentrations deviated from nominal. Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Groups/ Spacing of Exposure Levels N/A Only one concentration was used. Domain 4: Test Organism Test Organism Characteristics Low The exposure was via sediment. Domain 4: Test Organism Test Organism and Pretreatment Conditions Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Conditions Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and	8	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
Metric 6:Randomized AllocationLowResearchers did not report how organisms were allocated to study groups.Domain 3: Exposure CharacterizationMetric 7:Experimental System/Test Media PreparationMediumThe methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.Metric 8:Consistency of ExposureMediumThe study provided few details on exposure administration. AdministrationMetric 9:Measurement of Test Substance Metric 10:MediumWater concentrations were measured, but concentrations deviated from nominal. Concentration and Frequency Metric 11:Number of Exposure Groups/ Spacing of Exposure Groups/ N/AN/AOnly one concentration was used. Only one concentration was used. Spacing of Exposure Levels Metric 12:Test Organism Test organismN/AThe test organisms were wild caught. It was unclear at what development stage the exposure was initiated.Domain 4: Test Organism Metric 14:Acclimatization and Pretreatment Conditions Metric 15:LowThe test organisms were acclimatized. Conditions MediumThe study did not report whether test organisms were acclimatized. Conditions Medium		Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described. Metric 8: Consistency of Exposure Medium The study provided few details on exposure administration. Metric 9: Medium The study provided few details on exposure administration. Metric 10: Exposure Duration and Frequency Low The duration was appropriate, but lack of renewals led to fungal infections. Metric 11: Number of Exposure Groups/ N/A Only one concentration was used. Spacing of Exposure Levels N/A The exposure was via sediment. Domain 4: Test Organism Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Metric 7:Experimental System/Test Media PreparationMediumThe methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.Metric 8:Consistency of Exposure AdministrationMediumThe study provided few details on exposure administration.Metric 9:Measurement of Test Substance ConcentrationMediumWater concentrations were measured, but concentrations deviated from nominal.Metric 10:Exposure Duration and Frequency Metric 11:LowThe duration was appropriate, but lack of renewals led to fungal infections.Metric 12:Testing at or Below Solubility LimitN/AOnly one concentration was used.Domain 4: Test OrganismMetric 13:Test Organism CharacteristicsLowMetric 14:Acclimatization and Pretreatment ConditionsLowThe test organisms were wild caught. It was unclear at what development stage the exposure was initiated.Metric 14:Acclimatization and Pretreatment Conditions Metric 15:LowThe test organisms were acclimatized.Metric 14:Acclimatization and Pretreatment Conditions Metric 15:LowThe test organisms were stud did not report whether test organisms were acclimatized.	Domain 3: Exposure Cha	aracterization			
Metric 8: Consistency of Exposure Medium The study provided few details on exposure administration. Metric 9: Measurement of Test Substance Medium Water concentrations were measured, but concentrations deviated from nominal. Metric 10: Exposure Duration and Frequency Low The duration was appropriate, but lack of renewals led to fungal infections. Metric 11: Number of Exposure Groups/ N/A Only one concentration was used. Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit N/A Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.	ľ	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.
Administration Metric 9: Medium for Test Substance Medium Water concentrations were measured, but concentrations deviated from nominal. Metric 10: Exposure Duration and Frequency Low The duration was appropriate, but lack of renewals led to fungal infections. Metric 11: Number of Exposure Groups/ N/A Only one concentration was used. Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.
Concentration Metric 10: Exposure Duration and Frequency Low The duration was appropriate, but lack of renewals led to fungal infections. Metric 11: Number of Exposure Groups/ N/A Only one concentration was used. Spacing of Exposure Levels N/A The exposure was via sediment. Domain 4: Test Organism Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 13: Test Organism and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: N/A Only one concentration was used. Domain 4: Test Organism Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Pretreatment Low There were 10 organisms with 5 replicates for 50 total.		Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.
Spacing of Exposure Levels Metric 12: Spacing of Exposure Levels Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Dealiaste a per Group Medium There were 10 organisms with 5 replicates for 50 total.		Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.
Metric 12: Testing at or Below Solubility Limit N/A The exposure was via sediment. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		M-4.5 12.	Spacing of Exposure Levels	NT/A	
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		Metric 12:	Testing at or Below Solubility Limit	IN/A	The exposure was via sediment.
Metric 13: Test Organism Characteristics Low The test organisms were wild caught. It was unclear at what development stage the exposure was initiated. Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.	Domain 4: Test Organisr	m			
Metric 14: Acclimatization and Pretreatment Low The study did not report whether test organisms were acclimatized. Conditions Conditions Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.	C	Metric 13:	Test Organism Characteristics	Low	The test organisms were wild caught. It was unclear at what development stage the exposure was initiated.
Conditions Metric 15: Number of Organisms and Medium There were 10 organisms with 5 replicates for 50 total.		Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
Deplicates per Crown		Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.
Kephcaes per Group			Replicates per Group		
Domain 5: Outcome Assessment	Domain 5: Outcome Ass	sessment			
Metric 16: Adequacy of Test Conditions Low Inconsistencies in temperature were a concern.		Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.
Metric 17: Outcome Assessment Methodology Low The outcome assessment methodology was not clearly reported.		Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
Metric 18: Consistency of Outcome Medium Details regarding the execution of the study protocol for outcome assessment were lim-		Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-
Assessment ited.			Assessment		ited.
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Diethylhexyl Phthalate

HERO ID: 7978546 Table: 10 of 16

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	IVL, (1997). Overall Dura Aquatic (free Vertebrate; A Developmen Di-ethylhexy 7978546	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 7978546				
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, n=1.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	Additional Comments: This evaluation was for weight and % lipids.					
Overall Qualit	ty Detern	nination	Low			

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997) Overall Dura Aquatic (fre	The influence of sediment-associated pht ation: > 21 days; Exposure Duration: > 2 shwater); Sediment; Not determined by stu	halate esters (1 days udy authors (i	(DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. .e., chemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age: Health Outcome:	Vertebrate; A Developmen	Amphibian; <i>Rana arvalis</i> ; Embryo t/Growth			
Chemical: HERO ID:	Di-ethylhexy 7978546	yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design					
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.	
	Metric 9:	Administration Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.	
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m				
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.	
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Diethylhexyl Phthalate

HERO ID: 7978546 Table: 11 of 16

continued from previous page						
Study Citation:	IVL, (1997).	VL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Con	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
Domain 7. Dua Present	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, n=1.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This evaluati	ion was for weight and % lipids.				
Overall Quality Determination			Low			

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997) Overall Dura Aquatic (fre	. The influence of sediment-associated pht ation: > 21 days; Exposure Duration: > 2 shwater); Sediment; Not determined by stu	thalate esters (1 days udy authors (i	(DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. .e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo		
Health Outcome:	Developmen	nt/Growth		
Chemical: HERO ID:	7978546	yl phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.
Domain 2. Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail but the
		Preparation		experimental system was not adequately described.
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Duration was appropriate, but lack of renewals led to fungal infections.
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.
		Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.
		Replicates per Group		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
		Cont	tinued on nex	xt page

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Diethylhexyl Phthalate

HERO ID: 7978546 Table: 12 of 16

continued from previous page						
Study Citation:	IVL, (1997).	VL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.				
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Con	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	vsis				
Domain 7. Dua Present	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, n=1.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This evaluati	ion was for weight and % lipids.				
Overall Quality Determination			Low			

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997) Overall Dura Aquatic (fre	The influence of sediment-associated pht ation: > 21 days; Exposure Duration: > 2 shwater); Sediment; Not determined by stu	halate esters (1 days udy authors (i	(DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. .e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	7978546			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4.	Negative Controls	Hioh	Study authors reported using an appropriate concurrent pegative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups
	incure o.	Tundonin 200 Tunobulon	Low	researchers are not report now organisms were anotated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the
		Preparation		experimental system was not adequately reported.
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.
		Concentration		
	Metric 10:	Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.
		Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Matria 15.	Conditions	Madium	
	wieuric 15:	Replicates per Group	wiedium	There were 10 organisms with 5 replicates for 50 total.
		Replicates per Gloup		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
		Cont	tinued on nex	ct page

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 13 of 16

continued from previous page							
Study Citation:	IVL, (1997).	VL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	y authors (i	e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	7978546	7978546					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.			
Domain 7. Data Present	ation and Anal	vsis					
Domain 7. Data Present	Metric 21.	Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal			
	Mettie 21.	Statistical Methods	Low	of some samples due to infection was somewhat arbitrary.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad- pole.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	nts: This evaluation was for survival.						
Overall Quality Determination Low			Low				

Study Citation: Duration: Exposure Route, Media Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vertebrate; A	Amphibian; <i>Rana arvalis</i> ; Embryo			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)			
HERO ID:	7978546				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2. Test Design					
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
			2011		
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described	
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.	
		Administration			
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.	
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.	
		Spacing of Exposure Levels			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Demain 4. Test Oreania					
Domani 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	M 15	Conditions			
	Metric 15:	Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		ited.	
Continued on next page					

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 14 of 16

continued from previous page							
Study Citation:	IVL, (1997)	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	shwater); Sediment; Not determined by stud	ly authors (i	i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	7978546	7978546					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad- pole.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	: This evaluation was for survival.						
Overall Quality Determination Low							

Study Citation: Duration: Exposure Route, Media Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; A	Amphibian; <i>Rana arvalis</i> ; Embryo				
Health Outcome:	Mortality	1				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	7978546					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.		
Domain 2. Test Design						
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
			2011			
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of the test media were described in adequate detail, but the		
	Metric 8.	Consistency of Exposure	Medium	The study provided few details on exposure administration		
	Metile 0.	Administration	Wiedrum	The study provided few details on exposure duministration.		
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.		
Demain 4. Test Oreania						
Domani 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure		
	Matric 14	Acclimatization and Pratraatmont	Low	was initiated.		
	Metric 14.	Conditions	LOW	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Number of Organisms and	Medium	There were 10 organisms with 5 replicates for 50 total.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported		
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-		
	mente 10.	Assessment	moutuill	ited.		
		5000000				
		Cont	tinued on nex	t page		

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7978546 Table: 15 of 16

		contin	ued from p	previous page				
Study Citation:	IVL, (1997).	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	7978546							
Domain		Metric	Rating	Comments				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad- pole.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				
Additional Comments:	This evaluation was for survival.							
Overall Quali	ty Detern	nination	Low					

Study Citation: Duration: Exposure Route, Media, Path:	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome:	Vertebrate; A	Amphibian; <i>Rana arvalis</i> ; Embryo				
Chemical.	Di-ethylbey	vl phthalate (DFHP)				
HERO ID:	7978546	(DDTT)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	High	DEHP was analytically verified using GC-MS.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.6%.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent pegative control group		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
	Mettre 0.	Randomized / mocation	Low	Researchers and not report now organisms were anocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	Medium	The methods for preparation of test media were described in adequate detail, but the		
		Preparation		experimental system was not adequately described.		
	Metric 8:	Consistency of Exposure	Medium	The study provided few details on exposure administration.		
	Metric 9:	Measurement of Test Substance	Medium	Water concentrations were measured, but concentrations deviated from nominal.		
	Metric 10.	Concentration Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used		
	Methe 11.	Spacing of Exposure Levels	10/21	Only one concentration was used.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.		
Damain 4. Tart Orania						
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Matria 15.	Conditions Number of Organisms and	Madium			
	Metric 15:	Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.		
		1 ····· 1 · · · · · · · · · · · · · · ·				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-		
		Assessment		ited.		
	Continued on next page					

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Diethylhexyl Phthalate

HERO ID: 7978546 Table: 16 of 16

		contin	ued from p	previous page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, Rana arvalis. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Amphibian; <i>Rana arvalis</i>; Embryo ADME (biotransformation) Di-ethylhexyl phthalate (DEHP) 7978546 					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Temperature variations were evident.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not for control tadpoles.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Qualit	Overall Quality Determination Low					

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Study Citation:	Larson, P., T	Thuren, A. (1987). D-2-ethylhexylphthlalate	e inhibits the hate	ching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology			
Duration: Exposure Route, Media, Path:	and Chemistry $6(6)$:417-422. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A ADME (bio Di-ethylhexy 5508563	Amphibian; <i>Rana arvalis</i> ; Larvae transformation) yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	Metric 1:	Test Substance Identity	Low	The test substance was only identified by the chemical name. No other information was provided.			
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Kebo-Grave (Sweden), but the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups (i.e. all			
	incure ii		ingn	conditions equal except chemical exposure).			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.			
Domain 3: Exposure Cha	aracterization						
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set up and test media preparation methods were reported but did not completely account for physical-chemical properties.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using capillary gas chromatography. Measured concentrations were similar to nominal concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 8 treatment groups and two controls (sediment plus ethanol and water only). The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	No effects on biological responses were observed in the sediment plus ethanol or water only control groups, but the solvent concentration used was not provided.			
Domain 4: Test Organisi	n						
-	Metric 13:	Test Organism Characteristics	Medium	Frog eggs were collected from a pond in southern Sweden. Eggs were 2-3 days old. It was stated that the ponds received "organic contaminants only from airborne fallout" but no data was given to support this statement. Initial DEHP concentration in frog eggs was not determined.			
		Conti	nued on next pa	ge			

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Environmental Hazard Evaluation

HERO ID: 5508563 Table: 1 of 3

Study Citation: Larxon, P., Turren, A. (1987). D>-2ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicoli and Clemistry 6(6):417–422. Duration: Overall Duration: > 21 days Exposure Route, Media, Path: Aquatic (freshwater), Sediment: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rou Media, Path: Taxu, Species, Age: Vertebrate: Amphibian; Rana arradity, Larvae Exposure Comments Domain Metric (18: Comments) Metric (DEHP) Exposure array (Comments) Comments Domain Metric 14: Acclimatization and Pretreatment Conditions Low Conditions The study did not report whether test organisms were acclimatized and/or whether price trainment conditions were the same for control and exposed groups. Domain Metric 14: Acclimatization and Pretreatment Conditions Metric 17: Number of Organisms and Replicates per Group Metric 17: The numbers of dia ergina contraination existed and the control: The concol assessment methodology High The housing and environmental conditions were reported and sufficient to charact in the control assessment methodology Domain 6: Confounding / Variable Control Metric 18: Consistency of Outcome Assessment High The housing and environmental conditions were reported and success to report the intended outcome in for 60 days, and on information on aduces were sampled at the end of the exprime rand fo			contin	ued from previo	ous page			
Duration: Overall Duration: > 21 days: Exposure Duration: > 21 days Exposure Review Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rou Exposure Review Applicit (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rou Method Outcome Applicit (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rou Demain Metric (freshwater); Applicate (DEHP) ERO ID: Stoss503 Domain Metric 14: Acclimatization and Pretreatment Conditions Comments Conditions Metric 15: Number of Organisms and replicates per Group The study did not report whether stor organisms and replicates were reported and sufficient to character to take and replicates were used for each of the eight concentrations tested and the controls. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The howing and environmental conditions were reported and sufficient to character version accent set were assessment methodology and procease set were assessment methodology applicate were used for each of the eight concentration of Details of the experiment detarring the set of the experiment det	Study Citation:	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422						
Exposure Ronte, Media, Patti Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake ron Media, Patti Trax, Species, Age: Health Outcome: ADME (biotransformation) Vertebrate; Amphibian; Rana arvalis; Larvae Trax, Species, Age: Health Outcome: Chemical: Diettylhexyl phthalaet (DEHP) Vertebrate; Amphibian; Rana arvalis; Larvae Metric Metric (DEHP) Rating Comments Domain Metric 14: Conditions Acclimatization and Pretreatment Conditions Low The study did not report whether test organisms were acclimatized and/or whether pretreatment Conditions Domain 5: Outcome Assessment Metric 15: Number of Organisms and Replicates were used for each of the eight cancentrations tested and the controls. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High High The housing and environmenia conditions were reported and sefficient to the cipicates were used form each test vessel, honoganized, extracte with solutione assessment methodology addressed or roported, and uncomes were assessment methodology addressed or reported, and uncomes were assessment Domain 6: Confounding / Variable Comtrol Metric 18: Consistency of Outcome Assessment Low The study wid not provide enough information to all we a comparison of environment ran for 60 days, and no information on ware quality was provided. Domain 7: Data Presentation and Analysis Low The st	Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Media, Path: Taxa, Species, Age: Vertebrue; Amphibian: Rana arvalis; Larvae Health Outcome: ADME (biotransformation) Chemical: Di-ethylhexyl phthalade (DEHP) HERO ID: 550853 Domain Metric 14: Acclimatization and Pertreatment Conditions Metric 15: Number of Organisms and Replicates per Group Metric 16: Adequacy of Test Conditions Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Metric 18: Consistency of Outcome Assessment Metric 19: Confounding Variables in Test Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Metric 22: Reporting of Data Metric 22: Reporting of Data Metric 22: Reporting of Data Metric 23: Explanation of Unexpected Outcomes Metric 24: Statistical Methods Metric 25: Reporting of Data Metric 24: Statistical Methods Metric 25: Reporting of Data Metric 24: Statistical Methods Metric 25: Reporting of Data Metric 25: Reporting of Data Metric 25: Reporting of Data Metric 25: Reporting of Data Metric 25: Replanation of Unexpected Outcomes Metric 26: Replanation of Unexpected Outcomes Metric 27: Reporting of Data Metric 28: Explanation of Unexpected Outcomes Metric 29: Reporting of Data Metric 20: Replanation of Unexpected Outcomes Metric 20: Replanation of Unexpected Outcomes Metric 21: Statistical Methods Metric 21: Statistical Methods Metric 22: Reporting of Data Metric 23: Explanation of Unexpected Outcomes Metric 24: Reporting of Data Metric 25: Replanation of Unexpected Outcomes Met	Exposure Route,	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Vertebrate: Amphibian: Rana arvalis; Larvae Health Outcome ADDite (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5508563 Metric Domain Metric 14: Acclimatization and Pretreatment Conditions Low The study did not report whether test organisms were acclimatized and/or whether proceedings of test organisms and replicates were used of test organism and replicates were treported and sufficient to character ize toxicological effects. There were sproximately 100 eggs in each test vessel and replicates were used for each of the eight concentrations tested and the conduct ize toxicological effects. There were sproximately 100 eggs in each test vessel and replicates were used at ore can of the eight concentrations tested and the conduct ize toxicological effects. There were sproximately 100 eggs in each test vessel and replicates were used at of each of the eight concentrations tested and the conduct is the replicates were used at of con 12 h light 12 h dark photoperiod. Metric 17: Domain 5: Outcome Assessment High The housing and environmental conditions were reported and seemed to be conductive with solvents; 2.0-53 talphales were collected from each test vessel, homogenized, extracter with solvents; 2.0-53 talphales were collected from each test vessel, homogenized, extracter with solvents; 2.0-53 talphales were essent protocol were reported, and vitromised in the extra of the exprime test or study groups. Talpholes were sampled at the end of the exprime transformer from for 00 days, and no information in the study on outcomes study groups. Tale expriment conditions or othere non-tratation of DEHP	Media, Path:							
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Chemical: Di-ethylhexyl phhalate (DEHP) HERO ID: 5508563 Domain Metric Rating Comments Domain Metric 14: Acclimatization and Pretreatment Conditions Low The study did not report whether test organisms are acclimatized and/or whether preadment conditions were acclimatized and/or whether preadment and esposed groups. Metric 15: Number of Organisms and Replicates per Group Medium The numbers of test organisms and replicates were used for each of the eight concentrations tested and the controls. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High Metric 17: The housing and environmental conditions were reported and seemed to be conduced and the interded outcome or interval wave conducted a 50C or 12 hight: 12 hadx photoperiod. Metric 18: Consistency of Outcome Assessment High Metric 18: Consistency of Outcome Assessment High Metric 19: Constituence Confounding Variable Control Metric 19: Confounding Variables in Test Domain 6: Confounding Variable sin Test Metric 20: Low The study did not provide enough information to allow a comparison of environment conditions or other non-interation water quality was provided. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Statistical analysis was performed but not described adequately. Metric 21: Statisti	Health Outcome:	ADME (biot	transformation)					
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Metric 18: Consistency of Outcome Assessment High Details of the outcome assessment protocol were reported, and outcomes were assess consistently across study groups. Tadpoles were sampled at the end of the experimer determine tissue concentration of DEHP. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Low The study did not provide enough information to allow a comparison of environment conditions or other non-treatment-related factors across study groups. The experimer ran for 60 days, and no information on water quality was provided. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Statistical analysis was performed but not described adequately. Metric 22: Reporting of Data Low The concentrations in the tadpoles were shown in a figure. The actual concentration values are hard to interpret from the figure and in text. Only a range of values was gri across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all the treatment groups. The data was presented without measures of variabili across all th		Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. 20-25 tadpoles were collected from each test vessel, homogenized, extracted with solvents, and analyzed using capillary gas chromatography.			
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Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes. Additional Comments: None Medium		Metric 22:	Reporting of Data	Low	The concentrations in the tadpoles were shown in a figure. The actual concentration values are hard to interpret from the figure and in text. Only a range of values was given across all the treatment groups. The data was presented without measures of variability			
Additional Comments: None Overall Overlity Determination Medium		Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Averall Auglity Determination Medium	Additional Comments:	None						
	Overall Qualit	ty Dotorr	nination	Medium				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Health Outcome: Chemical: HERO ID:	Developmen Di-ethylhexy 5508563	ut/Growth yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by the chemical name. No other information was provided.		
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Kebo-Grave (Sweden), but the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups (i.e. all conditions equal except chemical exposure).		
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups were adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3 [,] Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set up and the test media preparation methods were reported but did not completely account for physical-chemical properties.		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using capillary gas chromatography. Measured concentrations were similar to nominal concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 8 treatment groups and two controls (sediment plus ethanol and water only). The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	No effects on biological responses were observed in the sediment plus ethanol or water only control groups, but the solvent concentration used was not provided.		
Domain 4: Test Organis	m					
C	Metric 13:	Test Organism Characteristics	Medium	Frog eggs were collected from a pond in southern Sweden. Eggs were 2-3 days old. It was stated that the ponds received "organic contaminants only from airborne fallout" but no data was given to support this statement. Initial DEHP concentration in frog eggs was not determined.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.		
		Cont	inued on next pa	ge		

HERO ID: 5508563 Table: 2 of 3

		contin	ued from previ	ous page				
Study Citation:	Larson, P., T and Chemist	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana arvalis; Embryo						
Health Outcome:	Developmen	nt/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5508563							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects. There were approximately 100 eggs in each test vessel and 5 replicates were used for each of the eight concentrations tested and the controls.				
Domain 5: Outcome As	ssessment							
	Metric 16:	Adequacy of Test Conditions	High	The housing and environmental conditions were reported and seemed to be conducive to health. The study was conducted at 5oC on 12 h light:12 h dark photoperiod.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. The hatching success of eggs were determined by counting tadpoles at 5, 12 and 30 days after exposure.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
Domain 7: Data Presen	tation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Descriptive statistics results (mean and standard deviation) were presented, but statistical tests to find significant differences among treatment groups were not conducted.				
	Metric 22:	Reporting of Data	Medium	Percent hatch of the moor frog eggs were presented for each treatment and control group via a figure. The counts of tadpoles recorded at 5, 12 and 30 days were not provided.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	The tadpole counts recorded at 5, 12 and 30 days were not provided. The percent hatch of the frog eggs were shown via a figure.							

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417.422						
Duration: Exposure Route, Media Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Di-ethylhexy 5508563	Amphibian; <i>Rana arvalis</i> ; Embryo yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by the chemical name. No other information was provided.			
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Kebo-Grave (Sweden), but the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%.			
Domain 2: Tast Dasian							
Domani 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups (i.e. all conditions equal except chemical exposure).			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set up and test media preparation methods were reported but did not completely account for physical-chemical properties.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using capillary gas chromatography. Measured concentrations were similar to nominal concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 8 treatment groups and two controls (sediment plus ethanol and water only). The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	No effects on biological responses were observed in the sediment plus ethanol or water only control groups, but the solvent concentration used was not provided.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Frog eggs were collected from a pond in southern Sweden. Eggs were 2-3 days old. It was stated that the ponds received "organic contaminants only from airborne fallout' but no data was given to support this statement. Initial DEHP concentration in frog eggs was not determined.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
		Conti	inued on next pa	ige			

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Environmental Hazard Evaluation

HERO ID: 5508563 Table: 3 of 3

		contin	ued from previ	ous page				
Study Citation:	Larson, P., 7 and Chemist	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthlalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:	· · · · · · · · · · · · · · · · · · ·							
Taxa, Species, Age:	Vertebrate; Amphibian; Rana arvalis; Embryo							
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5508563							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects. There were approximately 100 eggs in each test vessel and 5 replicates were used for each of the eight concentrations tested and the controls.				
Domain 5: Outcome As	ssessment							
	Metric 16:	Adequacy of Test Conditions	High	The housing and environmental conditions were reported and seemed to be conducive to health. The study was conducted at 5oC on 12 h light:12 h dark photoperiod.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. The hatching success of eggs were determined by counting tadpoles at 5, 12 and 30 days after exposure.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.				
Domain 6: Confoundin	9 / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
Domain 7: Data Presen	tation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Descriptive statistics results (mean and standard deviation) were presented, but statistical tests to find significant differences among treatment groups were not conducted.				
	Metric 22:	Reporting of Data	Medium	Percent hatch of the moor frog eggs were presented for each treatment and control group via a figure. The counts of tadpoles recorded at 5, 12 and 30 days were not provided.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	Mortality ou	tcome for percent hatch was presented in Fig	g 1, and tadpole	survival was reported in the results section.				

Overall Quality Determination

Medium

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Study Citation:	Zhang, Y., I	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis					
Duration	tadpoles. En	adpoles. Environmental Toxicology 33(1):112-121. Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route.	Aquatic (fre	shwater): Water: Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	i iquaite (iite						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana chensinensis; Larvae					
Health Outcome:	Developmen	nt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5493510						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	DEHP was identified as the test substance by name. No structure or CAS were reported.			
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Sigma Aldrich Corporation. It was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Purity was reported as 99% from the manufacturer.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A negative control was used and replicated.			
	Metric 5:	Negative Control Response	High	Control responses were adequate, and they were presented in Figure 1 and Table 2 on page 4/10.			
	Metric 6:	Randomized Allocation	Medium	Allocation of tadpoles was random.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Cr	Metric 7.	Experimental System/Test Media	Low	Concentrations were not measured during the study. Water was refreshed 50% every day			
	metric /.	Preparation	2011	and 100% every 3 days.			
	Metric 8:	Consistency of Exposure	Medium	Exposures appear consistent, but without measured concentrations, there is some uncer-			
		Administration		tainty.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and the solubility of DEHP and loss of the test substance may have been an issue. DEHP is poorly soluble in water and no solvent was used			
	Metric 10:	Exposure Duration and Frequency	High	Exposure was conducted until amphibians underwent metamorphosis, and days to meta- morphosis was calculated.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Treatments were spaced adequately to capture effects to metamorphosis: 0, 0.1, 1 and 10 umol/L DEHP.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations did not exceed the water solubility limit, but the test substance may also sorb to test vessels. Concentrations were not measured.			
Domain 4: Test Organis	Matria 12:	Test Organism Characteristics	Madiur	No continuo con esta Wildland in a circa forma and a state of the			
	Metric 13:	Test Organism Characteristics	Medium	No captive source was used. Wild breeding pairs of frogs were collected from China, and the tadpoles produced by those breeding pairs were used for the experiment.			
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions and animal husbandry are described and were comparable.			
	Metric 15:	Conditions Number of Organisms and	Medium	30 tadpoles per tank and 4 tanks per treatment were used.			
		Kephcales per Group					
	Continued on next page						

Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Zhang, Y., L tadpoles. En	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis adpoles. Environmental Toxicology 33(1):112-121.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana chensinensis; Larvae					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5493510						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	No water quality data was reported.			
	Metric 17:	Outcome Assessment Methodology	High	Sensitive endpoints were used that adequately captured effects to development and			
			8	growth. The endpoints included: Body weight, total length, hind limb length, and T0.5 (the time necessary for 50% of the tadpoles to reach Gosner stage 42 which is peak metamorphosis).			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups.			
Domain 6: Confounding	r / Variable Cou	atrol					
Domain 0. Comounding	Metric 10	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	Wieute 19.	Design and Procedures	Low	conditions or other non-treatment-related factors across study groups			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain /: Data Present	ation and Anal	ysis	TT: -1-				
	Metric 21:	Statistical Methods	High	Statistics are clearly described, and sufficient data were provided to conduct an indepen-			
	Metric 22:	Reporting of Data	High	Developmental/growth measurements for treatments and control are reported in Table 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes described.			
		r a martine competition of accounted	8				
Additional Comments:	Study exami length, body DEHP conce experiments	Study examined the effects of DEHP on amphibian metamorphosis. Endpoints relevant to development and growth included: Length of the hind limb, total length, body length, and T05 (the number of days for 50% of tadpoles to reach peak metamorphosis). Concentrations are nominal and were not verified. DEHP concentration may have been significantly lower than nominal if sorption to the test vessels occurred or if there were issues with the solubility. Other experiments have shown loss of the test substance over time.					

Overall Quality Determination

Medium

Study Citation:	Zhang, Y., I tadpoles. En	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis tadpoles. Environmental Toxicology 33(1):112-121.						
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (fre	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; A	Amphibian; Rana chensinensis; Larvae						
Health Outcome:	Endocrine							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5493510							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		Ţ					
	Metric 1:	Test Substance Identity	Low	DEHP was identified as the test substance by name only. No Structure or CASN were reported.				
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Sigma Aldrich Corporation, but it was not verified analyti- cally by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Purity was 99% as reported by the manufacturer.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	A negative control was used and replicated.				
	Metric 5:	Negative Control Response	Hign	Control responses were adequate and presented within narrative and Figures 2 and 3.				
	Metric 0.	Kandomized Anocation	Wedfulli	Anocation of tadpoles was fandom.				
Domain 3: Exposure Ch	aracterization							
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media Preparation	Low	Concentrations were not measured during the study. Water was refreshed 50% every day and 100% every 3 days.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures appear consistent, but without measured concentrations, there is some uncer- tainty.				
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and the solubility of DEHP and loss of the test substance may have been an issue. DEHP is poorly soluble in water and no solvent was used.				
	Metric 10:	Exposure Duration and Frequency	High	Exposure was conducted until amphibians underwent metamorphosis, and days to meta- morphosis was calculated.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Treatments were spaced adequately to capture effects to metamorphosis: 0, 0.1, 1 and 10 umol/L DEHP.				
	Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations did not exceed the water solubility limit, but the test substance may also sorb to test vessels. Concentrations were not measured.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	No captive source was used. Wild breeding pairs of frogs were collected from China, and the tadpoles produced by those breeding pairs were used for the experiment.				
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions and animal husbandry are described and were comparable.				
	Metric 15:	Conditions Number of Organisms and	Medium	30 tadpoles per tank and 4 tanks per treatment were used. For histology, 6 tadpoles from				
		Kephcates per Group		כמכוו ווכמנווכות שכוב עצבע.				

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

HERO ID: 5493510 Table: 2 of 3

		contin	ued from previ	ous page				
Study Citation:	Zhang, Y., I tadpoles. En	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis tadpoles. Environmental Toxicology 33(1):112-121.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana chensinensis; Larvae						
Health Outcome:	Endocrine							
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5493510							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	No water quality data was reported.				
	Metric 17:	Outcome Assessment Methodology	High	Endocrine effects were examined using quantitative analysis of follicle size and shape (Figure 3) from the thyroid gland (using histology). Gonads were also examined qualitatively using histology. Sex ratios of tadpoles were calculated (Figure 5).				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.				
Domain 6: Confounding	y / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	veie						
	Metric 21:	Statistical Methods	High	Statistics are clearly described, and sufficient data were provided to conduct an indepen- dent statistical analysis.				
	Metric 22:	Reporting of Data	High	Thyroid gland morphology is reported for treatments and control in Figure 3.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes described.				
Additional Comments:	Study exam ment/metam (qualitative) vessels occu	Study examined the effects of DEHP on amphibian metamorphosis. Several endpoints relevant to the endocrine system (which controls develop- ment/metamorphosis) were measured. Apical endpoints evaluated in this form include: thyroid gland histology results (quantitative) and gonadal histology (qualitative). Concentrations are nominal and were not verified. DEHP concentration may have been significantly lower than nominal if sorption to the test vessels occurred or if there were issues with the solubility. Other experiments have shown loss of the test substance over time.						

Overall Quality Determination

Medium

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HERO ID: 5493510 Table: 3 of 3

Study Citation:	Zhang, Y., I	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis						
Duration: Exposure Route, Media. Path:	tadpoles. En Overall Dur Aquatic (fre	tadpoles. Environmental Toxicology 33(1):112-121. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana chensinensis; Larvae						
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Receptor binding/ regulation of receptor activity						
Chemical:	Di-ethylhex	yl phthalate (DEHP)						
HERO ID:	5493510							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce	— • • • •	-					
	Metric 1:	Test Substance Identity	Low	DEHP was identified as the test substance by name only; no structure or CASN were reported.				
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Sigma Aldrich Corporation. It was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Purity was 99% as reported from the manufacturer.				
Domain 2: Test Design	Matria 4.	Na actions Countralia	II: -h					
	Metric 4: Matria 5:	Negative Controls	High	A negative control was used and replicated.				
	Metric 5.	Randomized Allocation	Medium	Allocation of tadpoles was random				
	Metale 0.	Randomized / mocarion	Weatum					
Domain 3: Exposure Ch	aracterization							
-	Metric 7:	Experimental System/Test Media Preparation	Low	Concentrations were not measured during the study. Water was refreshed 50% every day and 100% every 3 days.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures appear consistent, but without measured concentrations, there is some uncer- tainty.				
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and the solubility of DEHP and loss of the test substance may have been an issue. DEHP is poorly soluble in water and no solvent was used.				
	Metric 10:	Exposure Duration and Frequency	High	Exposure was conducted until amphibians underwent metamorphosis, and days to meta- morphosis was calculated.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Treatments were spaced adequately to capture effects to metamorphosis: 0, 0.1, 1 and 10 umol/L DEHP.				
	Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations did not exceed the water solubility limit, but the test substance may also sorb to test vessels and concentrations were not measured.				
Domain 4: Tast Organia	m							
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Medium	No captive source was used, wild breeding pairs of frogs were collected from China, and the tadpoles produced by those breeding pairs were used for the experiment				
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions and animal husbandry are described and were comparable.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	30 tadpoles per tank and 4 tanks per treatment were used. For hormone measurements and gene expression, n=5 replicates per treatment were used.				
		rr						

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 5493510 Table: 3 of 3

		contin	ued from previ	ous page			
Study Citation:	Zhang, Y., I tadpoles, En	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in Rana chensinensis					
Duration:	Overall Dura	Inverall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Rana chensinensis; Larvae					
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Receptor binding/ regulation of receptor activity					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)	C				
HERO ID:	5493510						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	No water quality data was reported.			
	Metric 17:	Outcome Assessment Methodology	High	Mechanistic endpoints evaluated in this form included: Thyroid hormone concentrations (T3/T4) and gene expression of sex steroid relevant genes.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistics are clearly described, and sufficient data were provided to conduct an indepen- dent statistical analysis.			
	Metric 22:	Reporting of Data	High	T4 and T3 concentration are reported for treatments and control in Figure 4. Gene expression is reported in Figure 6.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes described.			
Additional Comments:	Study exam ment/metam pression of s nominal if s time.	ined the effects of DEHP on amphibian n orphosis) were measured. Mechanistic end sex steroid relevant genes. Concentrations a orption to the test vessels occurred or if the	netamorphosis. points evaluate re nominal and re were issues v	Several endpoints relevant to the endocrine system (which controls develop- d in this form included: Thyroid hormone concentrations (T3/T4) and gene ex- were not verified. DEHP concentration may have been significantly lower than with the solubility. Other experiments have shown loss of the test substance over			

Overall Quality Determination

Medium

Study Citation:	Henderson, l	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed				
Duration: Exposure Route, Modia, Path:	zooplankton Overall Dura Aquatic (fres	rich in wax esters. Comparative Biochemi ation: > 21 days; Exposure Duration: > 2 shwater); Food/Diet; Dietary	istry and Phys 1 days	siology - Part C: Comparative Pharmacology 74(2):325-330.		
Taxa, Species, Age:	Vertebrate: F	ish: Salmo gairdneri: Adult				
Health Outcome:	Nutritional &	Metabolic				
Chemical:	Di-ethylhexy	/l phthalate (DEHP)				
HERO ID:	5353221					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].		
	Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
Domain 21 1000 Doorgi	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Cha	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure	Low	Details of exposure administration were not reported.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	N/A	Only one exposure dose was used.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via diet.		
Domain 4: Tast Organiza						
Domain 4. Test Organisi	Metric 13.	Test Organism Characteristics	Medium	There are uncertainties about the age of test organisms		
	Metric 14.	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms		
	Metric 15:	Conditions Number of Organisms and	Low	The number of test organisms and replicates were not reported.		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.		
		Cont	inued on nex	t page		

HERO ID: 5353221 Table: 1 of 4

		conti	nued from p	previous page			
Study Citation:	Henderson, zooplankton	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed zooplankton rich in wax esters. Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology 74(2):325-330.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Salmo gairdneri; Adult						
Health Outcome:	Nutritional d	& Metabolic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5353221						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
	Assessment						
Domain 6: Confounding	g / Variable Co	ntrol					
L. L	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.			
		Design and Procedures	8				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7. Data Present	ation and Anal	veis					
Domain 7. Data Present	Metric 21.	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Peporting of Data	Medium	Data for exposure related findings were shown for each treatment and control group			
	Metric 22.	Reporting of Data	Medium	Results were also described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluat	ion was for lipid content.					
Overall Qualit	tv Deterr	nination	Low				

Study Citation:	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed				
Duration:	zooplankton Overall Dura	rich in wax esters. Comparative Biochemi ation: > 21 days: Exposure Duration: > 2	istry and Phys	siology - Part C: Comparative Pharmacology 74(2):325-330.	
Exposure Route,	Aquatic (free	shwater); Food/Diet; Dietary			
Media, Path:					
Taxa, Species, Age:	Vertebrate; I	Fish; Salmo gairdneri; Adult			
Health Outcome:	Mechanistic	-Liver toxicology			
Chemical:	Di-ethylhexy	/l phthalate (DEHP)			
HERO ID:	5555221				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate	
	incure I.	Test Substance Taching	mgn	(DEHP)].	
	Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."	
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization		Ŧ		
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8:	Consistency of Exposure	Low	Details of exposure administration were not reported.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one exposure dose was used.	
	M 10	Spacing of Exposure Levels	NT/ A		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Medium	There are uncertainties about the age of the test organisms.	
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates were not reported.	
Domain 5: Outcome As	sessment				
2 ontain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
		Cont	inued on per	zt nage	
Continued on next page					

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HERO ID: 5353221 Table: 2 of 4

continued from previous page						
Study Citation:	Henderson,	R. J., Sargent, J. R. (1983). Studies on the	effects of di	i-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed		
	zooplankton	rich in wax esters. Comparative Biochemi	stry and Phys	siology - Part C: Comparative Pharmacology 74(2):325-330.		
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (freshwater); Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Salmo gairdneri; Adult					
Health Outcome:	Mechanistic	Mechanistic-Liver toxicology				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5353221					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
	Assessment					
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures	e			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
Domain / Data Present	Metric 21.	Statistical Methods	Low	Statistical analysis was performed but not described adequately		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group		
	Wieute 22.	Reporting of Data	Wiedium	Results were also described in the text.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion was for enzymes.				
Overall Quality Determination		Low				

Study Citation:	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed					
Duration: Exposure Route, Media. Path:	zooplankton Overall Dura Aquatic (fres	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo gairdneri; Adult				
Health Outcome:	Hepatic/Live	er				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5353221					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].		
	Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.		
Domain 2. Test Design						
2 omani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure	Low	Details of exposure administration were not reported.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	N/A	Only one exposure dose was used.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.		
Domain 4: Test Organisi	n					
	Metric 13:	Test Organism Characteristics	Medium	There are uncertainties about the age of the test organisms.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates were not reported.		
Domain 5: Outcome Ass	sessment					
Domain 5. Outcome Also	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.		
	Metric 17:	Outcome Assessment Methodology	Low	The methods used to assess liver weights were not reported.		
		Cont	inued on nex	t page		

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HERO ID: 5353221 Table: 3 of 4

		contin	ued from p	previous page			
Study Citation:	Henderson, zooplankton	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed zooplankton rich in wax esters. Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology 74(2):325-330.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo gairdneri; Adult					
Health Outcome:	Hepatic/Live	er					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5353221						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Low	Since methods were not reported, it is uncertain whether there was consistency.			
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Liver weight data was shown in Table 1, and results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and measures of variability were shown with the data.			
Additional Comments:	This evaluat	ion is for the liver weight assessment.					
Overall Quali	Overall Quality Determination Low						

Study Citation:	Henderson, I	R. J., Sargent, J. R. (1983). Studies on the	e effects of di	-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed
Duration: Exposure Route,	Overall Dura Aquatic (fres	rich in wax esters. Comparative Biochem: tion: > 21 days; Exposure Duration: > 2 shwater); Food/Diet; Dietary	1stry and Phy 1 days	stology - Part C: Comparative Pharmacology 74(2):325-330.
Media, Path: Taya Species Age:	Vertebrate: F	ish: Salmo gairdneri: Adult		
Health Outcome	Development	t/Growth		
Chemical:	Di-ethylhexy	phthalate (DEHP)		
HERO ID:	5353221	r		
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].
	Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
2 chian 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
	, . <i>.</i> .			
Domain 3: Exposure Cha	aracterization		т	
	Metric /:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Low	Details of exposure administration were not reported.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/	N/A	Only one exposure dose was used.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via diet.
		· · · · ·		
Domain 4: Test Organisi	m Matri: 12	Test Ores size Charles in the	M. 1	
	Metric 13:	Lesi Organism Characteristics	Medium	I nere are uncertainties about the age of the test organisms.
	metric 14:	Conditions	nıgn	An pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates were not reported.
Domain 5: Outcome Ass	resement			
Domain 5. Outcome Ass	Metric 16.	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
			••••••	
		Cont	inued on ney	kt page

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HERO ID: 5353221 Table: 4 of 4

		contin	ued from p	previous page			
Study Citation:	Henderson,	R. J., Sargent, J. R. (1983). Studies on the	effects of d	i-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout (Salmo gairdnerii) fed			
D d	zooplankton	zooplankton rich in wax esters. Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology 74(2):325-330.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; Salmo gairdneri; Adult						
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5353221						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.			
		Design and Procedures	e				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group			
	Methe 22.	Reporting of Data	Low	but results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Low				

Study Citation:	Mehrle, P. N	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace				
Duration	Substances i	n Environmental Health 10:519-524. ation: > 21 days: Exposure Duration: > 21 d	avs			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study aut	thors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	1					
Taxa, Species, Age:	Vertebrate; I	Fish; Salmo gairdneri; Embryo				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	791717					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
U	Metric 4:	Negative Controls	Low	It was unclear if the author used a negative control or a solvent control.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of the test media were described		
		Preparation		in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered		
	Matria 0.	Administration	Iliah	consistently across study groups.		
	Metric 9:	Concentration	High	Researchers used radiolabeled chemical to monitor concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	A flow-through design with an appropriate duration was reported.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.		
		Spacing of Exposure Levels	C			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.		
Domain 4: Test Organis	m Matria 12:	Test Organism Characteristics	Lliah	The test successions many adapted is described and some above a described from a well 11		
	Metric 15:	A colimatization and Protroctmont	Low	The study did not report whether test experience were obtained from a reliable source.		
	Meuric 14.	Conditions	LOw	The study did not report whether lest organisms were acclimatized.		
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (3) were reported and sufficient to charac-		
		Replicates per Group		terize toxicological effects.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health		
	methe 10.	racquies of fest conditions	Weatum	though few details were provided.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.		
		Continu	ued on next pa	ge		

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Environmental Hazard Evaluation

		continu	ued from previ	ous page			
Study Citation:	Mehrle, P. N	A., Mayer, F. L. (1976). Di-2-ethylhexyl ph	thalate: Residu	e dynamics and biological effects in rainbow trout and fathead minnows. Trace			
Derestiene	Substances i	Substances in Environmental Health 10:519-524.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquetic (freehwater): Water: Not determined by study outborg (i.e., shemical of interact in exposure water, but unable to determine exact untake route)						
Exposure Koule,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Pain:							
Taxa, Species, Age:	Vertebrate; Fish; Salmo gairdneri; Embryo						
Chamical	Mortanty Di athulhaw	Mortality					
	Di-ethylnexyl phthalate (DEHP)						
HERU ID;	/91/1/						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Hıgh	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	TT' 1				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain /: Data Present	ation and Anal	lysis	τ				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	Medium				

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Study Citation:	Mehrle, P. N	I., Mayer, F. L. (1976). Di-2-ethylhexyl ph	nthalate: Residu	e dynamics and biological effects in rainbow trout and fathead minnows. Trace
Duration:	Substances in Overall Dura	n Environmental Health 10:519-524. tion: > 21 days: Exposure Duration: > 21 d	davs	
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	uthors (i.e., chem	nical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Vertebrate; F	ish; <i>Salmo gairdneri</i> ; Embryo		
Health Outcome:	Di athulhavi	t/Growth		
HERO ID:	791717	1 pitilalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce		6	
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
e	Metric 4:	Negative Controls	Low	It was unclear if the author used a negative or a solvent control.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of the test media were described
		Preparation		in adequate detail.
	Metric 8:	Consistency of Exposure Administration	Hıgh	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Researchers used radiolabeled chemical to monitor concentrations.
	Metric 10:	Exposure Duration and Frequency	High	A flow-through design with an appropriate duration was reported
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.
		Spacing of Exposure Levels	6	
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.
Domain 4: Test Organis	m			
0	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15.	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates (3) were reported and sufficient to charac-
	Methe 15.	Replicates per Group	Wiedrum	terize toxicological effects.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
		Contir	nued on next pa	ge

Environmental Hazard Evaluation

		continu	ued from previ	ious page	
Study Citation:	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace				
Duration:	Substances in Environmental Health 10:519-524. Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., cher	nical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo gairdneri; Embryo			
Health Outcome:	Developmen	t/Growth			
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	791717				
Domain	Metric Rating Comments				
Domain 6: Confoundin	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presen	tation and Anal	vsis			
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.	
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability	
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.	
Additional Comments:	This evaluat	on form was added for development/growth	endpoint with	rainbow trout embryos.	

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Mehrle, P. N	I., Mayer, F. L. (1976). Di-2-ethylhexyl ph	nthalate: Residu	e dynamics and biological effects in rainbow trout and fathead minnows. Trace
Duration	Substances i	n Environmental Health 10:519-524. ation: > 21 days: Exposure Duration: > 21 d	davs	
Exposure Route,	Aquatic (free	shwater); Water: Not determined by study at	uthors (i.e., chem	nical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	1			
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo gairdneri; Embryo		
Health Outcome:	ADME (biot	ransformation)		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	791717			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	It was unclear if the author used a negative control or a solvent control.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Researchers used radiolabeled chemical to monitor concentrations by GLC.
	Metric 10:	Exposure Duration and Frequency	High	A flow-through design with an appropriate duration were reported.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.
Domain 4: Test Organisi	m Matria 12	Test Organism Characteristics	Ul ah	The test approxime many adapted is dependent of the dependence of the second state
	Metric 14:	A colimatization and Pretreatment	Low	The study did not report whether test organisms were continued from a reliable source.
	Methe 14.	Conditions	Low	The study did not report whether lest organisms were accimianzed.
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (3) were reported and sufficient to charac-
		Replicates per Group		terize toxicological effects.
Domain 5: Outcome Ass	accmant			
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
		Contir	nued on next pa	ge

Environmental Hazard Evaluation

		continu	ued from previ	ous page		
Study Citation:	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace					
Duration:	Substances in Environmental Health 10:519-524. Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo gairdneri; Embryo				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	791717					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	y / Variable Con Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High	There were no reported differences among the study groups in environmental conditions.		
Domain 7: Data Present	ation and Anal	vsis	Ingn	There were no unreferees among groups that could influence the outcome assessment.		
Domain 7. Data Frederic	Metric 21	Statistical Methods	Low	Statistical analysis was not conducted, which is typical for BCF assessments		
	Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes, not for controls		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		

Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gor	such, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
Duration:	organisms. E Overall Dura	Environmental Toxicology and Chemistry attion: 0 - 4 days (0-96h); Exposure Duration	14(9):1569-15 on: 0 - 4 days	574. (0-96h)
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	X7 / 1 / T			
Taxa, Species, Age:	Vertebrate; F	ish; Salmo mykiss; Juvenile		
Health Outcome:	Mortality	d abthalate (DEUD)		
Chemical:	Di-ethylnexy	I phthalate (DEHP)		
Demain	1321990	Matria	Dating	Comments
Domain 1: Test Substan		Metric	Kating	Comments
Domain 1. Test Substant	Metric 1.	Test Substance Identity	Low	Test substance nomenclature was reported without CASRN
	Metric 2:	Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available
	Medic 2.		Low	batches. The manufacture name and batch number not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	At least 95% purity was reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure Ch	Metric 7	Experimental System/Test Media	Medium	The experimental system was well described. However, the headspace or the measures
	Wette 7.	Prenaration	Wiedium	taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.
		Administration	0	
	Metric 9:	Measurement of Test Substance	High	Sample extracts were analyzed by gas chromatography at the start and end of the test.
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed
	whether 11.	Spacing of Exposure Levels	Ingn	Exposure levels were appropriate. A range minding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organisi	n Maria 12		т	
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation for the test was reported.
	Metric 15:	Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test
		Replicates per Group		vessel.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
		Cont	inued on nex	t page

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Diethylhexyl Phthalate

		conti	nued from p	revious page	
Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic	
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-1	574.	
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	on: 0 - 4 days	(0-96h)	
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vertebrate; Fish; Salmo mykiss; Juvenile				
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1321996				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent across groups.	
		Assessment			
Domain 6: Confounding	v / Variabla Co	ntrol			
Domain 0. Comountuing	Matric 10	Confounding Variables in Test	High	The environmental conditions were consistent earces groups	
	Methe 19.	Design and Procedures	Ingn	The environmental conditions were consistent across groups.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.	
Domain 7: Data Procent	tation and Anal	voie			
Domain 7. Data Fresent	Metric 21.	ysis Statistical Methods	High	Statistical matheds were performed and described	
	Metric 22:	Penorting of Data	Medium	Only treatment endpoints were reported	
	Metric 22.	Explanation of Unavaorated Outcomes	Lich	No unaverse ted outcomes were reported.	
	Metho 25:	Explanation of Onexpected Outcomes	nıgii	no unexpected outcomes were reported.	
Additional Comments:	None				
Overall Qualit	ty Detern	nination	High		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Norrgren, L.	, Blom, A., Andersson, P. L., Boerjeson, F.	H., Larsson, J., D.	G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol			
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days	har). Aquate Ecosystem Hearth and Management 2(3).311-317.			
Exposure Route,	Aquatic (free	shwater); N/A (e.g., injection); Injection					
Media, Path:	W (1 (1						
Taxa, Species, Age: Hoolth Outcomo:	Vertebrate; I	Pisn; Salmo Salar; Juvenile Biomarkers (apposure and effect)					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	5646979	()					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		-				
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.			
	Metric 2:	Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but the DEHP was not verified analytically.			
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%			
Domain 2: Test Design							
6	Metric 4:	Negative Controls	High	Positive (estradiol in peanut oil) and carrier (peanut oil) controls were used.			
	Metric 5:	Negative Control Response	High	The control responses in the presence/absence of VTG in plasma were reported and adequate (Figure 2, Page 6/8).			
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.			
Domain 3: Exposure Ch	aracterization						
Domain of Enposite of	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors adequately reported preparation of injections and handling of fish. There was a minor omission on whether handling alone due to intraperitoneal injection administration caused any unwarranted stress or morbidity and/or mortality in fish.			
	Metric 8:	Consistency of Exposure Administration	Low	Authors indicate that injections per day were between 2-4, and also report two different durations for exposures (14 and 17 days).			
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations in the intraperitoneal injection preparations were not measured. DEHP values in intraperitoneal injection (80 and 160 mg/kg body weight) were only reported as nominal.			
	Metric 10:	Exposure Duration and Frequency	Medium	A 17 day exposure with daily administration of the test compound could result in vitel- logenin (VTG) production. However, it is not clear how many injections were adminis- tered since the authors reported that organisms were injected intraperitoneally with test solution 2-4 times during the 17-day exposure period. The authors did not explain why they administered 2-4 injections.			
	Metric 11:	Number of Exposure Groups/	Medium	Exposure groups were 80 and 160 mg DEHP/kg body weight as well as controls. How- ever no instification was provided for the injection concentrations			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via injection.			
Domain 4: Test Organis	m Matri 12	Track Operations Characteristic	TT' 1				
	Metric 13:	lest Organism Characteristics	High	The source and age of the juveniles were reported. The species (Salmo salar) was appro- priate for the intended outcome of the study.			
		Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 5646979 Table: 1 of 2

		contin	ued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa Species Age:	Norrgren, L. and PCB) or Overall Dura Aquatic (free Vertebrate: F	, Blom, A., Andersson, P. L., Boerjeson, H. a sexual differentiation in juvenile Atlantic sa ation: 11 - 21 days; Exposure Duration: 11 - shwater); N/A (e.g., injection); Injection	, Larsson, J., D. almon (Salmo sa 21 days	G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylpheno alar). Aquatic Ecosystem Health and Management 2(3):311-317.
Health Outcome	Mechanistic	-Biomarkers (exposure and effect)		
Chemical:	Di-ethylhexy	vl phthalate (DEHP)		
HERO ID:	5646979	() produce () 2222)		
Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate for one week to the rearing conditions in a flow- through (river water provided) system with 50L per 5 animals (7.5 g each).
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 fish per group were administered the intraperitoneal injections. The 5 fish per group were maintained in separate tanks. There were no replicates.
Domain 5: Outcome As	ssessment			
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (plasma collection and western blot analysis of vitellogenin) was reported and adequate for the outcome of interest (determine the con centrations of vitellogening in plasma). While vitellogenin was detected in the positive control group, it was not detected in the DEHP (80 and 160 mg/kg body weight) group
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appeared to be assessed similarly across the treatment and control group
Domain 6: Confoundin	σ / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing was reported to indicate that environmental variables confounded the test re- sults, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presen	tation and Anal	veic		
Domain 7. Data 1 lesen	Metric 21:	Statistical Methods	N/A	This study relied on the presence/absence of a vitellogenin band on western blots.
	Metric 22:	Reporting of Data	Medium	This study relied on the presence/absence of vitellogenin on western blots. In the re- sults section, the authors described that vitellogenin was not detected in plasma after intraperitoneal injections of DEHP.
	Metric 23:	Explanation of Unexpected Outcomes	High	In the results section, the authors described that vitellogenin was not detected in plasm after intraperitoneal injections of DEHP. The authors thought that the findings were surprising since estrogenic chemicals such as DEHP had been shown in previous inves tigations to cause proliferation of MCF-7 cells in vitro. Moreover, the authors explaine that the size and dose regime of the experiment may have affected the production of vitellogening and indicated that an alternative biomarker used for the detection of estro genic chemicals such as DEHP could be the production of zona radiata protein (ZRP).

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Diethylhexyl Phthalate

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Study Citation:	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration	: 11 - 21 days	1			
Exposure Route,	Aquatic (freshwater); N/A (e.g., injection); Injectio	n				
Media, Path:						
Taxa, Species, Age:	Vertebrate; Fish; Salmo Salar; Juvenile					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5646979					
Domain	Metric Rating Comments					
Overall Quali	ty Determination	Medium				

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Study Citation: Duration: Exposure Route, Media, Path:	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); N/A (e.g., injection); Injection							
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Salmo Salar; Juvenile						
Health Outcome:	Endocrine							
Unemical:	5646070	I phthalate (DEHP)						
	5040979							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce Matric 1:	Test Substance Identity	Low	No CAS or structure was provided. Chemical identified by nomenalature				
	Metric 2:	Test Substance Source	Low	No CAS of structure was provided. Chemical identified by nomenciature.				
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%				
	incure 5.		mgn					
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Positive (estradiol in peanut oil) and carrier (peanut oil) controls were used.				
	Metric 5:	Negative Control Response	High	The control responses in presence/absence of VTG in plasma are reported in fig 2 (Page 6/8).				
	Metric 6:	Randomized Allocation	Low	random allocation was not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors adequately reported preparation of injections and handling of fish. No survival metrics are reported for the 17-day study.				
	Metric 8:	Consistency of Exposure Administration	Low	Authors indicate that injections per day were between 2-4 and also report two different durations for exposures (14 and 17 days).				
	Metric 9:	Measurement of Test Substance	Low	Values are reported as nominal.				
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	A 17 day exposure with daily administration of the test compound could result in VTG production.				
	Metric 11:	Number of Exposure Groups/	Medium	No justification was provided for the injection concentrations (80, 160 mg/kg, DEHP).				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Injections.				
Domain 4: Test Organis	m							
C C	Metric 13:	Test Organism Characteristics	High	The source and age of larvae were reported.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems with 50L per 5 animals (7.5 g each).				
	Metric 15:	Number of Organisms and Replicates per Group	Low	These injections were conducted with triplicate groups (N=14)				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.				
	Metric 17:	Outcome Assessment Methodology	Uninformative	VTG was not detected from fish administered 80, 160 mg/kg DEHP daily for 17 days.				
			Continued on next page	·				

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Diethylhexyl Phthalate

		(continued from previous pa	ige			
Study Citation:	Norrgren, L. and PCB) or	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317					
Duration:	Overall Dura	Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater): N/A (e.g., injection): Injection					
Media, Path:	$\mathbf{T}_{\mathbf{r}}$						
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo Salar; Juvenile					
Health Outcome:	Endocrine						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5646979						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	They outcomes appeared to be assessed similarly across the treatment and control			
		Assessment		groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Medium	Nothing was reported to indicate that environmental variables confounded the test re-			
		Design and Procedures	_	sults, however, the authors did not report these conditions for the study.			
	Metric 20:	Outcomes Unrelated to Exposure	Low	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	This study relied on the presence/absence of VTG on western blots.			
	Metric 22:	Reporting of Data	Medium	This study relied on the presence/absence of VTG on western blots.			
	Metric 23:	Explanation of Unexpected Outcomes	N/A	This study relied on the presence/absence of VTG on western blots.			
Additional Comments:	None						
Overall Quali	ty Detern	nination	Uninformative				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Norrgren, L.	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol					
	and PCB) on	and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Roule, Madia Dath:	Aquatic (free	snwater); Food/Diet; Dietary					
Taxa, Species, Age:	Vertebrate: F	Fish: Salmo Salar: Larvae					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	/l phthalate (DEHP)					
HERO ID:	5646979						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.			
	Metric 2:	Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but DEHP was not verified analytically.			
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.			
	Metric 5:	Negative Control Response	High	The control responses in liver somatic index (LSI) of juvenile salmon were reported and adequate (Table 2, Page 5/8).			
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.			
Domain 3: Exposure Ch	aracterization		TT' 1				
	Metric /:	Experimental System/Test Media Preparation	High	Authors adequately reported preparation of the treated diet as well as handling of the compound in section 2.2 (page 3/8).			
	Metric 8:	Consistency of Exposure	High	There is no evidence suggesting that dietary treatments were not administered equally among groups			
	Metric 9:	Measurement of Test Substance	Low	DEHP in experimental diet was not measured. DEHP values in diet (300 and 1500			
		Concentration		mg/kg food) were reported as nominal and based on an estimated 2% body weight basis.			
	Metric 10:	Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad differentiation in fry within Atlantic Salmon.			
	Metric 11:	Number of Exposure Groups/	Medium	The authors reported exposing organisms to 300 and 1500 mg DEHP/kg of food. How-			
		Spacing of Exposure Levels		ever, the authors presented no justification for the concentrations used in the feeding study.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposures were via dietary treatments.			
Domain 4: Test Organisi	III Matria 12:	Test Organism Characteristics	Uiah	The course and age of large ware reported. The species (Column solar) was super-			
	Metric 15.	Test Organism Characteristics	nigii	for the intended outcome of the study.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replication was reported but each treatment had 200 individuals.			
		Tephones per Group					

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Diethylhexyl Phthalate

Study Citation: Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Aquatic (freshwater); Food/Diet; Dietary Media. Path: Taxa, Species, Age: Vertebrate; Fish; Salmo Salar; Larvae **Health Outcome:** Development/Growth Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5646979 Domain Metric Rating Comments Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Housing was adequate, however, the authors did not provide or record environmental parameters during the study. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology [liver dissection and weighing to calculate the liver somatic index (LSI): liver weight/total body weight X 100] was appropriate for the outcome reported (DEHP effect on liver development/growth). Metric 18: Consistency of Outcome High They outcomes appeared to be assessed similarly across the treatment and control groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium Nothing was presented to indicate that differences were due to attrition or animal health. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High One-way analysis of variance (ANOVA) was used to determine if there was a difference in the liver somatic index (LSI) between the control group and the exposed groups. Metric 22: Reporting of Data High The sample size, mean LSI value and standard deviation across treatment groups were presented in Table 2 (Page 5/8). Metric 23: Explanation of Unexpected Outcomes Medium Variance was presented in Table 2. The sample sizes are not equal between treatment and control groups. Additional Comments: The aim of the present study was to perform in vivo studies on Atlantic salmon to evaluate the effects of DEHP exposure (oral/dietary or intraperitoneal) to study feminization in terms of skewed sex ratios and vitellogenin (VTG) production. Because VTG is stored in the liver, liver development (liver somatic index) was also examined. This form was used to evaluate liver developmental/growth effects of DEHP dietary exposure in salmon fry.

Overall Quality Determination

Medium

Study Citation:	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCP) on cavual differentiation in invanila. Atlantic calman (Salma calar). A quartic Ecosystem Health and Management 2(2):211–217				
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	ation: > 21 days; Exposure Duration: > 21 shwater); Food/Diet; Dietary	days	nar). Aquate Ecosystem nearin and Management 2(5).511-517.	
Taxa, Species, Age:	Vertebrate: F	Fish: Salmo Salar: Larvae			
Health Outcome:	Reproductiv	e/Teratogenic			
Chemical:	Di-ethylhexy	vl phthalate (DEHP)			
HERO ID:	5646979				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.	
	Metric 2:	Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but DEHP was not verified analytically.	
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%.	
Domain 2: Test Design					
6	Metric 4:	Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.	
	Metric 5:	Negative Control Response	High	The control responses (positive and solvent control) for sex determination were adequate and reported in Figure 1 (Page 5/8).	
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Ch	aracterization				
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	Authors adequately reported preparation of the treated diet as well as handling of the compound in section 2.2 (page 3/8).	
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence suggesting that dietary treatments were not administered equally among groups.	
	Metric 9:	Measurement of Test Substance	Low	DEHP in experimental diet was not measured. DEHP values in diet (300 and 1500 mg/kg food) were reported as nominal and based on an estimated 2% body weight basis.	
	Metric 10:	Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad development and differentia- tion within Atlantic Salmon.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors reported exposing organisms to 300 and 1500 mg DEHP/kg of food. How- ever, the authors presented no justification for the concentrations used in the feeding study.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposures were via dietary treatments.	
Domain 4: Test Organis					
	Metric 13:	Test Organism Characteristics	High	The source and age of larvae were reported. The species (Salmo salar) was appropriate for the intended outcome of the study.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replication was reported, but each treatment had 200 individuals.	
Domain 5: Outcome Ass	sessment				

Continued on next page ...

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Diethylhexyl Phthalate

			F			
Study Citation:	Norrgren, L.	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol				
Duration: Exposure Route, Media Path	and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management $2(3):311-317$. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary					
Tava Snecies Age	Vertebrate: F	Fish: Salmo Salar: Larvae				
Health Outcome	Reproductiv	e/Teratogenic				
Chemical.	Di-ethylbey	vl phthalate (DFHP)				
HFRO ID.	5646979	yi philialate (DEIII)				
Domain	5010777	Matric	Rating	Comments		
Domain	Matria 16:	Adequacy of Test Conditions	Medium	Housing was adequate howayar the authors did not provide or record environmental		
	Weute 10.	Adequacy of Test Conditions	Medium	parameters during the study.		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (gonad classification as males those with thin colorless gonads and as females those with pinkish 'bulbs'; sex was confirmed by light microscopic examination) was reported and appropriate for the intended outcome of interested (DEHP effects on sex determination).		
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appeared to be assessed similarly across the treatment and control groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Nothing was reported to indicate that environmental variables confounded the test re- sults, however, the authors did not report these conditions for the study.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	A chi-square test for association was used to determine if there was a correlation be- tween the number of females in each group and diet.		
	Metric 22:	Reporting of Data	Low	Figure 1 presented the number of females among treatment and control groups. The total number of organisms (males plus females) per treatment group should have been reported. The authors only reported a range of the total number of individuals per treatment group to be between 82 and 184, which a wide range and it is impossible to determine the actual ratio of males to females.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The number of phenotypic females were presented but no other information was reported.		
Additional Comments:	The aim of the study femini index) was a sex ratio out	he present study was to perform in vivo studi ization in terms of skewed sex ratios and vite also examined. This form was used to evalue come displayed in Fig. 1	ies on Atlantic s ellogenin (VTG) ate DEHP dietar	almon to evaluate the effects of DEHP exposure (oral/dietary or intraperitoneal) to production. Because VTG is stored in the liver, liver development (liver somatic y exposure on the gonad development in salmon fry. This form also assesses the		

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Norrgren, L. and PCB) or Overall Dura Aquatic (free	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Reproductive Di-ethylhexy 5646979	Fish; <i>Salmo Salar</i> ; Larvae e/Teratogenic yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. Chemical identified by nomenclature.			
	Metric 2:	Test Substance Source	Low	Source as listed but not verified analytically.			
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.			
	Metric 5:	Negative Control Response	High	The control responses (positive and solvent control) for sex determination are reported in Figure 1 (Page 5/8).			
	Metric 6:	Randomized Allocation	Low	random allocation was not reported.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	Authors adequately reported preparation of the treated diet and handling of the com- pound were reported in section 2.2 (page 3/8).			
	Metric 8:	Consistency of Exposure Administration	High	The dietary treatments were administered equally among groups.			
	Metric 9:	Measurement of Test Substance	Low	Values are reported as nominal and based on an estimated 2% body weight basis.			
	Metric 10:	Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad differentiation within At- lantic Salmon.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors presented no justification for the concentrations used in the feeding study.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary treatments.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source and age of larvae were reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replication was reported but each treatment had 200 individuals.			
Domain 5: Outcome Ass	esement	······································					
Domain 5. Outcome As:	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.			
	Metric 17:	Outcome Assessment Methodology	Medium	The methods assessment was appropriate for the outcomes reported (sex determination). Survival among treatment groups was not reported.			
		Conti	nued on next pa	ge			

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Aquatic (freshwater); Food/Diet; Dietary Media, Path: Taxa, Species, Age: Vertebrate; Fish; Salmo Salar; Larvae **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5646979 Domain Metric Rating Comments Metric 18: Consistency of Outcome High They outcomes appeared to be assessed similarly across the treatment and control groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Medium Nothing was reported to indicate that environmental variables confounded the test re-**Design and Procedures** sults, however, the authors did not report these conditions for the study. Metric 20: Outcomes Unrelated to Exposure Low Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival. Domain 7: Data Presentation and Analysis Statistical Methods Chi-squared analysis was reported for determining significant differences in HSI. Metric 21: High Metric 22: Reporting of Data Low Figure 1 presented the number of females among treatments and control groups. The total number of animals represented was not reported for each group, which should have been reported. Metric 23: Explanation of Unexpected Outcomes Low The number of phenotypic females were presented but no other information was reported.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Norrgren, L.	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in invenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317					
Duration: Exposure Route, Modia Path:	Overall Dura Aquatic (free	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary					
Taxa. Species. Age:	Vertebrate: F	Fish: Salmo Salar: Larvae					
Health Outcome:	Hepatic/Live	er					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5646979						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. Chemical identified by nomenclature.			
	Metric 2:	Test Substance Source	Low	Source as listed but not verified analytically.			
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.			
	Metric 5:	Negative Control Response	High	The control responses in HSI are reported in Table 2 (Page 5/8).			
	Metric 6:	Randomized Allocation	Low	random allocation was not reported.			
Domain 2. Evenance Ch	anatonization						
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Media	Uich	Authors adapted to managed an antiparticle of the twested dist and headling of the same			
	Metric 7.	Preparation	nigii	pound were reported in section 2.2 (page 3/8)			
	Metric 8:	Consistency of Exposure	High	The dietary treatments were administered equally among groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Values are reported as nominal and based on an estimated 2% body weight basis.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad differentiation within At- lantic Salmon.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors presented no justification for the concentrations used in the feeding study.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary treatments.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source and age of larvae were reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replication was reported but each treatment had 200 individuals.			
Domain 5: Outcome As	sessment		Madian				
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.			
	Metric 17:	Outcome Assessment Methodology	High	The methods assessment was appropriate for the outcomes reported (Hepato somatic index).			
Continued on next page							

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Diethylhexyl Phthalate

		contir	ued from previo	us page		
Study Citation:	Norrgren, L. and PCB) or	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon (Salmo salar). Aquatic Ecosystem Health and Management 2(3):311-317				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Food/Diet; Dietary	•			
Media, Path:		•				
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo Salar; Larvae				
Health Outcome:	Hepatic/Live	er				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5646979					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	They outcomes appeared to be assessed similarly across the treatment and control		
		Assessment		groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Medium	Nothing was reported to indicate that environmental variables confounded the test re-		
		Design and Procedures	Ŧ	sults, however, the authors did not report these conditions for the study.		
	Metric 20:	Outcomes Unrelated to Exposure	Low	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	ANOVA was reported for determining significant differences in HSI.		
	Metric 22:	Reporting of Data	Medium	The sample number, HSI value and SD are presented in Table 2 (Page 5/8).		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variance was presented in Table 2. The sample sizes are not equal between treatment and control groups.		
Additional Comments:	None					
Overall Quality Determination Medium						

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar)						
Duration: Exposure Route, Media Path	dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary						
Taxa. Species. Age:	Vertebrate: F	Fish: Salmo salar: Larvae					
Health Outcome:	Development/Growth						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5678430						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The test substance identified by chemical name. No other information was provided.			
	Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).			
	Metric 5:	Negative Control Response	High	The biological response of control was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2. Europuna Ch	anastanization						
Domain 5: Exposure Cr	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.			
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks, and were fed (approximately 2% of their body weight daily) four times per day.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of exposure groups and the spacing of exposure levels were not conducive to assess growth effects. There was no significant difference in weight among treatment groups.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.			
		C	ontinued on next page .				
Diethylhexyl Phthalate

		CO	ntinued from previous	page				
Study Citation: Duration: Exposure Route, Media, Path:	Norman, A., dietary expo Overall Dura Aquatic (free	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary						
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; Salmo salar; Larvae						
Health Outcome:	Development/Growth							
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	5678430							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group. Growth measurements (length and weight) were conducted 4 months post-exposure. Housing and environmental conditions during this post-exposure period were not mentioned.				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology only partially addressed or reported the intended outcome(s) of interest. Growth measurement methodology was not described well.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Growth measurements were taken 4 months post-exposure in all study groups and control.				
Domain 6: Confoundin	g / Variable Co	ntrol						
Domain o. Comoundan	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups especially during the 4 month post-exposure.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.				
Domain 7: Data Presen	tation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed. In the methods, it was stated that one way ANOVA was used to examine whether weight and length differed significantly between groups. However, in the results section, statistical results of length data were not provided.				
	Metric 22:	Reporting of Data	Uninformative	Data presentation was inadequate (e.g., the report does not differentiate among findings in multiple treatment groups).				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	The growth a and control.	neasurements were not provided for treatmen	t groups and control. No	significant differences in weight were observed among treatment groups				

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary Vertebrate; Fish; <i>Salmo salar</i> ; Larvae				
Health Outcome:	Hepatic/Live	er			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)			
HERO ID:	5678430				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The test substance identified by chemical name. No other information was provided.	
	Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design	Matria 4:	Nagatiya Controla	Uigh	Study outhors reported using an emperation consument acceptive control enough (i.e. all	
	Meuric 4.	Negative Controls	rigi	conditions equal except chemical exposure).	
	Metric 5:	Negative Control Response	High	The biological response of the control was reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 2. Expedites Ch	anastanization				
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks and were fed (approximately 2% of their body weight daily) four times per day.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of exposure groups and the spacing of exposure levels were not conducive to measure the effects on the liver. There were no significant effects on hepatosomatic index among treatment groups.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organis	m		TT' 1		
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14:	Acclimatization and Pretreatment	Hıgh	The test organisms were acclimatized to test conditions, and all pretreatment conditions	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.	
		Repleates per Group		t · · · · · ·	

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

... continued from previous page

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary Vertebrate; Fish; <i>Salmo salar</i> ; Larvae Hepatic/Liver Di-ethylhexyl phthalate (DEHP) 5678430				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during the exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group. Mortality was recorded 4 months post-exposure. Housing and environmental conditions during this post-exposure period were not mentioned.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology was clearly reported.	
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were not clearly reported.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups especially during the 4 months post-exposure, after which mortality was recorded.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. In the methods, it was stated that chi square test was used to examine whether mortality differed significantly between groups.	
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. It was only stated that mortality in all groups was low (3-4%).	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	No significat	nt difference in hepatosomatic index among tre	eatment groups was re	ported.	

Overall Quality Determination

Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Norman, A., dietary expos Overall Dura Aquatic (fres Vertebrate; F Reproductive	Börjeson, H., David, F., Tienpont, B., Nor sed to di-2-ethylhexyl phthalate (DEHP) du tion: > 21 days; Exposure Duration: > 21 shwater); Food/Diet; Dietary "ish; <i>Salmo salar</i> ; Larvae e/Teratogenic	rgren, L. (2 ring early li days	2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) fe. Archives of Environmental Contamination and Toxicology 52(2):235-242.
Chemical: HERO ID:	Di-ethylhexy 5678430	l phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substanc	e			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No other information was pro- vided.
	Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	The biological response of the control was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Cha	racterization			
Domain 5. Exposure Cha	Metric 7	Experimental System/Test Media	Hioh	The experimental system and methods for preparation of the test media were described
		Preparation		in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks and were fed (approximately 2% of their body weight daily) four times per day.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organisn	n			
6	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media Path:	Norman, A., dietary expo Overall Dura Aquatic (free	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate: F	Sish: Salmo salar: Larvae				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	5678430					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during the exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group. The histopathology of gonads was conducted 4 months post -exposure. Housing and environmental conditions during this post-exposure period were not mentioned.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (histological evaluation of gonads) addressed or reported the intended outcome(s) of interest.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. The mortality in all groups was low- 3-5% at 4 months post exposure.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data Present	Metric 21:	Statistical Methods	High	Statistical analysis was performed and described well. Sex ratios, intersex ratios, and precocious ratios were analyzed using Fisher's exact test for significant differences between groups.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Fig 2). Pictures of histopathology results from control fish and fish exposed to the highest DEHP concentration were provided (Figures 3-5). Numbers of fish presenting ovotestis (intersex) were counted for each treatment.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	The histolog evaluations.	cical evaluation included gonadal different	tiation and p	presence of ovo-testis. Findings were supported by data and photos of histological		

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chomical	Norman, A., dietary expo Overall Dura Aquatic (free Vertebrate; F ADME (biot	Börjeson, H., David, F., Tienpont, B., No sed to di-2-ethylhexyl phthalate (DEHP) du ation: > 21 days; Exposure Duration: > 21 shwater); Food/Diet; Dietary Fish; Salmo salar; Larvae transformation)	rrgren, L. (2 aring early li days	2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) fe. Archives of Environmental Contamination and Toxicology 52(2):235-242.
HERO ID:	5678430	(DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No other information was pro- vided.
	Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design				
2 onium 21 1000 2 oorgin	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	The biological response of the control was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
	, . ,.			
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Media	Uich	The experimental system and methods for properties of the test medic years described
	Meuric 7.	Preparation	nigii	in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks and were fed (approximately 2% of their body weight daily) four times per day.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organis	m			
rest orgunis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route,	Norman, A., dietary expo Overall Dura Aquatic (free	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	ish; Salmo salar; Larvae					
Health Outcome:	ADME (biol	ransformation)					
UEDO ID.	Di-ethylnexy	(DEHP)					
HERO ID:	30/8430						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for fish tissue concentrations.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 1).			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) for fish tissue concentrations.			
Additional Comments:	Fish tissue c	concentration values were provided on a view of the term of te	wet-weight an orted in the p	nd lipid normalized basis. Also, fish tissue concentrations of of mono-2-ethyl hexyl paper.			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path:	Norman, A., dietary expos Overall Dura Aquatic (fres	Börjeson, H., David, F., Tienpont, B., Norr sed to di-2-ethylhexyl phthalate (DEHP) dur ation: > 21 days; Exposure Duration: > 21 d shwater); Food/Diet; Dietary	gren, L. (2007). Studies ing early life. Archives of lays	of uptake, elimination, and late effects in atlantic salmon (Salmo salar) f Environmental Contamination and Toxicology 52(2):235-242.
Taxa, Species, Age:	Vertebrate; F	Sish; Salmo salar; Larvae		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	5678430			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce		_	
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No other information was pro- vided.
	Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	The biological response of control was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 2. Expedium Ch	anatanization			
Domain 5: Exposure Cr	Metric 7	Experimental System/Test Media	High	The experimental system and methods for preparation of the test media ware described
	Weute 7.	Preparation	Ingn	in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks and were fed (approximately 2% of their body weight daily) four times per day.
	Metric 11:	Number of Exposure Groups/	Uninformative	The number of exposure groups and the spacing of exposure levels were not conducive to assess lethal effects. Mortality was low (3.4%) in all groups
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
		· · · ·		
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

... continued from previous page

Study Citation:	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon (Salmo salar) dietary exposed to di-2-ethylbexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate: F	Fish: Salmo salar: Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5678430					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during exposure seemed to be conducive for the maintenance of health. Mortality was recorded 4 months post-exposure. Housing and environmental conditions during this post-exposure period were not mentioned.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups especially during the 4 months post-exposure, after which mortality was recorded.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. In the methods, it was stated that chi square test was used to examine whether mortality differed significantly between groups.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. It was only stated that mortality in all groups was low (3-4%).		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Mortality wa	as low in all groups (3-4%).				
Overall Quali	ty Detern	nination	Uninformative			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Dumpert, K.	., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for de	termining the embryotoxic effects of environmental chemicals. Ecotoxi-			
Duration:	cology and I Overall Dura	Environmental Safety 8(1):55-74. ation: > 21 days; Exposure Duration: Not-re	eported				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	31448						
Domain	Metric Rating Comments						
Domain 1. Test Substan	Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure was provided in Figure 1.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Both a solvent-control and a water-only control were utilized in this experiment.			
	Metric 5:	Negative Control Response	Uninformative	In Experiment I, the methyl alcohol control was observed to cause embryo toxicity (perhaps by promoting the growth of bacteria), thus making it impossible to differentiate any effects due to DEHP alone. Experiment II addressed these concerns by removing the use of the alcohol control.			
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.			
Domain 3: Exposure Ch	oracterization						
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media	Medium	Authors utilized components in the experimental set-up most likely containing DEHP			
		Preparation		(plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, preparation of the stock solutions of DEHP was not adequately described.			
	Metric 8:	Consistency of Exposure	Low	Details of DEHP dosing and renewal of the basins were limited.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	DEHP concentrations in water were measured via GC-FID and were measured imme- diately after dosing and 12 days after dosing. Di-n-hexyl phthalate was utilized as a standard solution. No details were provided on reproducibility or recovery.			
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was not explicitly stated, but as is common in amphibian devel- opmental studies, lasted for the duration of development (or time to complete metamor- phosis).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five exposure concentrations ranged from 0.1-20 ppm DEHP. One basin containing between 70-83 larvae was utilized per test concentration and per control group.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	Reported solubility for DEHP is approximately 0.3 ppm. Authors report test concentrations above this limit, but they report utilizing methanol as the solvent to increase solubility of DEHP.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source of breeding stock was described.			
		С	Continued on next page .				

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Diethylhexyl Phthalate

Study Citation:

Exposure Route,

Media, Path:

Chemical:

HERO ID:

Domain

Duration:

... continued from previous page Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxicology and Environmental Safety 8(1):55-74. Overall Duration: > 21 days; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Taxa, Species, Age: Vertebrate; Amphibian; Xenopus laevis; Larvae **Health Outcome:** Development/Growth Di-ethylhexyl phthalate (DEHP) 31448 Metric Rating Comments Metric 14: Acclimatization and Pretreatment Low The acclimatization period was not described by authors (unclear when dosing com-Conditions menced). Metric 15: Number of Organisms and Low There was one basin per test concentration containing between 70-83 larvae per basin. Replicates per Group Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Authors do not report pH, DOC, or DO for water in test basins. Metric 17: Outcome Assessment Methodology Medium Authors cite Nieuwkoop and Faber 1956 for outcome assessment methodology. Details of the histological examination were not well described. Metric 18: Consistency of Outcome Low Timing and frequency of the execution of outcome assessment protocol were not re-Assessment ported.

Domain 6: Confounding	/ Variable Cont	rol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Uninformative	Bacterial infection in the solvent control and the DEHP control basin but not in the water control basin confounded interpretation of developmental endpoints from this experiment.
	Metric 20:	Outcomes Unrelated to Exposure	Uninformative	Authors report the presence of bacterial infection in the solvent control and all DEHP treatment basins, and state they could not distinguish whether embryotoxic effects were due to DEHP treatment or presence of bacteria in the basins.
Domain 7: Data Presenta	tion and Analys	sis		
	Metric 21:	Statistical Methods	Uninformative	Statistics were not reported for this data.
	Metric 22:	Reporting of Data	Uninformative	Developmental endpoint data were not reported for each treatment group, nor were they distinguished between solvent control and DEHP treatments.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report variability.

Additional Comments: In Experiment I, the methyl alcohol control was observed to cause embryo toxicity (perhaps by promoting the growth of bacteria), thus making it impossible to differentiate any effects due to DEHP alone.

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 31448 Table: 2 of 4

Study Citation:	Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxi- cology and Environmental Safety 8(1):55-74				
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	ition: > 21 days; Exposure Duration: Not-re shwater); Water; Not determined by study au	ported thors (i.e., chemical of i	nterest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae			
Health Outcome:	Developmen	t/Growth			
HERO ID:	31448	(DEFF)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure was provided in Figure 1.	
	Metric 2:	Test Substance Source	Low	The source was not reported.	
	Metric 3:	Test Substance Purity	Low	The purity was not reported.	
Domain 2. Test Design					
Domani 2. Test Design	Metric 4:	Negative Controls	High	Both a Holtfreter solution control and a water-only control were utilized in this experi- ment.	
	Metric 5:	Negative Control Response	Medium	Time from egg to full developed frog is stated in Table 2 (variation among test organ- isms within the control basins was not provided).	
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.	
Domain 3: Exposure Ch	aracterization		т		
	Metric 7:	Preparation	Low	Authors utilized components in the experimental set-up most likely containing DEHP (Plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, preparation of the stock solutions of DEHP was not adequately described. Concentrations were not reported for Experiment 2.	
	Metric 8:	Consistency of Exposure	Low	Details of DEHP dosing and renewal of the basins were limited.	
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	DEHP concentrations in water were not reported for Experiment 2. DEHP concentration differences between the control basin and DEHP basins were provided, but concentrations of the basins were not provided	
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration lasted for the duration of development (time to metamorphosis), and was thus different across treatments.	
	Metric 11:	Number of Exposure Groups/	N/A	DEHP (10 ppm) was added either once or at repeated intervals in the DEHP basins.	
		Spacing of Exposure Levels			
	Metric 12:	Testing at or Below Solubility Limit	Low	Reported solubility for DEHP is approximately 0.3 ppm. Authors tested 10 ppm DEHP without reporting measured concentrations and did not utilize solvent to increase DEHP solubility in the water.	
Domain 4: Test Organis	m				
_ main rost organis	Metric 13:	Test Organism Characteristics	High	The source of breeding stock was described.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The acclimatization period was not described by authors (unclear when dosing com- menced).	
		C	ontinued on next page		

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxicology and Environmental Safety 8(1):55-74. **Duration:** Overall Duration: > 21 days; Exposure Duration: Not-reported **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Vertebrate; Amphibian; Xenopus laevis; Larvae **Health Outcome:** Development/Growth Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 31448 Domain Metric Rating Comments Number of Organisms and Metric 15: Low The number of test organisms that were exposed to DEHP per basin was not clear. Authors state a total of 360 larvae hatched from 4 basins and then state 30 tadpoles per Replicates per Group basin remained. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Authors do not report pH, DOC, or DO for water in test basins. Authors state loading 6 L basins with 200 eggs per basin with low hatching success. Metric 17: Outcome Assessment Methodology Medium Authors cite Nieuwkoop and Faber 1956 for outcome assessment methodology. Details of the histological examination were not well described. Metric 18: Consistency of Outcome Low The timing and frequency of the execution of outcome assessment protocol were not reported. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low There was high attrition of larvae from eggs, and it was not reported in which tanks the Design and Procedures attrition was the greatest. Outcomes Unrelated to Exposure Low Metric 20: Authors state high attrition of test organisms prior to conducting the experiment. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Uninformative No statistics were performed for data in Table 2. Metric 22: Reporting of Data High Time to development was shown in Figure 2. Metric 23: Explanation of Unexpected Outcomes Low Authors did not report variability.

Additional Comments: None

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 31448 Table: 3 of 4

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxi- cology and Environmental Safety 8(1):55-74. Overall Duration: > 21 days; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae Mortality Di-ethylhexyl phthalate (DEHP) 31448				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure	
	Matric 2.	Test Substance Source	Low	was provided in Figure 1.	
	Metric 2: Metric 3:	Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Both a Holtfreter solution control and a water-only control were utilized in this experi- ment.	
	Metric 5:	Negative Control Response	Medium	Survival rate of the controls is stated in Table 2.	
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.	
Domain 3: Exposure Ch	aracterization Metric 7: Metric 8: Metric 9: Metric 10: Metric 11: Metric 12:	Experimental System/Test Media Preparation Consistency of Exposure Administration Measurement of Test Substance Concentration Exposure Duration and Frequency Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit	Low Low Low N/A Low	 Authors utilized components in the experimental set-up most likely containing DEHP (Plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, preparation of the stock solutions of DEHP was not adequately described. Concentrations were not reported for Experiment 2. Details of DEHP dosing and renewal of the basins were limited. DEHP concentrations in water were not reported for Experiment 2. DEHP concentration differences between the control basin and DEHP basins were provided, but concentrations of the basins were not provided. The exposure duration lasted for the duration of development (time to metamorphosis), and was thus different across treatments. DEHP (10 ppm) was added either once or at repeated intervals in the DEHP basins. Reported solubility for DEHP is approximately 0.3 ppm. Authors tested 10 ppm DEHP without reporting measured concentrations, and they did not utilize a solvent to increase DEHP solubility in the water. 	
Domain 4: Test Organisi	m Metric 13: Metric 14:	Test Organism Characteristics Acclimatization and Pretreatment Conditions	High Low Continued on next page	The source of breeding stock was described. The acclimatization period was not described by authors (unclear when dosing com- menced).	

Diethylhexyl Phthalate

		con	tinued from previou	s page			
Study Citation:	Dumpert, K. cology and F	Dumpert, K., Zietz, E. (1984). Platanna (Xenopus laevis) as a test organism for determining the embryotoxic effects of environmental chemicals. Ecotoxi- cology and Environmental Safety 8(1):55-74.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: Not-repo	orted				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study authors	ors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:		· · ·					
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	31448						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms that were exposed to DEHP per basin was not clear. Au- thors state a total of 360 larvae hatched from 4 basins and then state 30 tadpoles per basin remained.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Authors do not report pH, DOC, or DO for water in test basins. Authors state loading 6 L basins with 200 eggs per basin with low hatching success.			
	Metric 17:	Outcome Assessment Methodology	Low	Details for assessing survival were not described (unclear whether dead larvae were removed daily or on a regular basis).			
	Metric 18:	Consistency of Outcome Assessment	Low	Timing and frequency of the execution of outcome assessment protocol were not re- ported.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There was high attrition of larvae from eggs prior to initiating the experiment, and it was not reported in which tanks the attrition was the greatest.			
	Metric 20:	Outcomes Unrelated to Exposure	Low	Authors state high attrition of test organisms prior to conducting the experiment.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	This was a limit test.			
	Metric 22:	Reporting of Data	High	Survival rate after 200 days was shown in Figure 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report variability.			

Additional Comments: None

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 31448 Table: 4 of 4

Study Citation:	Dumpert, K	., Zietz, E. (1984). Platanna (Xenopus laevis)) as a test organism for de	termining the embryotoxic effects of environmental chemicals. Ecotoxi-		
Duration: Exposure Route, Media, Path:	cology and l Overall Dur Aquatic (fre	Sology and Environmental Salety 8(1):55-74. Overall Duration: > 21 days; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhex	yl phthalate (DEHP)				
HERO ID:	31448					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure was provided in Figure 1.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity was not reported.		
Domain 2: Test Design						
Domain 21 Test Design	Metric 4:	Negative Controls	High	Both a solvent-control and a water-only control were utilized in this experiment.		
	Metric 5:	Negative Control Response	Uninformative	Authors state survival in control basins was approximately 30%. This would indicate approximately 70% mortality of frog embryos in the control groups.		
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors utilized components in the experimental set-up most likely containing DEHP (Plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, preparation of the stock solutions of DEHP was not adequately described.		
	Metric 8:	Consistency of Exposure	Low	Details of DEHP dosing and renewal of the basins were limited.		
	Metric 9:	Administration Measurement of Test Substance	Medium	DEHP concentrations in water were measured via GC-FID and were measured imme-		
		Concentration		diately after dosing and 12 days after dosing. Di-n-hexyl phthalate was utilized as a standard solution. No details were provided on reproducibility or recovery.		
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration differed across treatments and was the duration of time it took for amphibians to complete development (time to complete metamorphosis).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five exposure concentrations ranged from 0.1-20 ppm DEHP. One basin containing between 70-83 larvae was utilized per test concentration and per control group.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	Reported solubility for DEHP is approximately 0.3 ppm. Authors report test concentra- tions above this limit but report utilizing methanol as the solvent to increase solubility of DEHP.		
Domain 4: Test Organis	m					
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The source of the breeding stock was described		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The acclimatization period was not described by authors (unclear when dosing com- menced).		
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was one basin per test concentration containing between 70-83 larvae per basin.		
		Replicates per Group	ontinued on next page .			

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 31448 Table: 4 of 4

		0	ontinued from previous	page		
Study Citation:	Dumpert, K	., Zietz, E. (1984). Platanna (Xenopus laevis) Environmental Safety 8(1):55-74.) as a test organism for de	etermining the embryotoxic effects of environmental chemicals. Ecotoxi-		
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: Not-re	ported			
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)		
Media, Path:			ζ, j			
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhex	yl phthalate (DEHP)				
HERO ID:	31448	-				
Domain		Metric Rating Comments				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Authors do not report pH, DOC, or DO for water in test basins. The low level of survival in all groups indicate that 6 L may not be an adequate size for the number of embryos per basin.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not described (i.e. were dead embryos checked for daily and removed from basins). The endpoint was described as survival, not mortality.		
	Metric 18:	Consistency of Outcome Assessment	Low	The timing and frequency of the execution of outcome assessment protocol were not reported. Authors stated all animals were euthanized at 3 months after hatching, but the determination of mortality prior to this point was not described.		
Domain 6: Confounding	a / Variable Co	ntrol				
Domain 0. Comounding	Metric 10.	Confounding Variables in Test	Uninformative	Bacterial infection in the calvent control and the DEHP control basin but not in the		
	Wietite 17.	Design and Procedures	Chimorniative	water control basin could confound interpretation of mortality in this study.		
	Metric 20:	Outcomes Unrelated to Exposure	Uninformative	Authors report the presence of bacterial infection in the solvent control and in all the DEHP treatment basins, and they state they could not distinguish whether embryotoxic effects were due to DEHP treatment or the presence of bacteria in the basins.		
Domain 7: Data Present	tation and Anal	lysis				
	Metric 21:	Statistical Methods	Uninformative	Statistics were not reported for this data.		
	Metric 22:	Reporting of Data	Uninformative	Survival data were not reported for each treatment group but instead was presented as an average across all DEHP groups.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report variability.		
Additional Comments:	Metric 23: In Experime to differentia	Explanation of Unexpected Outcomes ent I, the methyl alcohol control was observed ate any effects due to DEHP alone.	Low to cause embryo toxicity	Authors did not report variability. (perhaps by promoting the growth of bacteria), thus making it impossible		

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Duration: Vorall Duration: > 21 days Exposure Route, Aquatic (freshowater): Sediment: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Pauli: Taxa, Species, Age: Invertebrate: Arthropods: Aesina ap: Larvae Health Outcome Obermical: Domain Metric 0: Domain Metric 0: Domain Metric 1: Test Substance Metric 0: Metric 2: Test Substance Metric 0: Metric 2: Test Substance Metric 0: Metric 2: Negative Controls Metric 2: Negative Controls Metric 2: Negative Controls Metric 2: Negative Control Megone Metric 3: Negative Control Megone Metric 4: Negative Control Megone Metric 4: Negative Control Megone Metric 4: Negative Control Megone Metric 5: Negative Control Megone Metric 6: Randomized Allocation Metric 7: Negative Control Megone Metric 7: Negative Control Megone Metric 6: Randomized Allocation Metric 7: Negative Control Megone Metric 8: Negative Control Megone Metric 9: Metric 9	Study Citation:	Woin, P., La	rsson, P. (1987). Phthalate esters reduce pre	edation efficiency of dragonf	ly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination
Exposure Route, Hola, Paula Aquatis (Hersi-water): Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Aquatis (Hersi-water): Arthropods: Acsima pr; Larvae Exposure (Intermined the Paula) Application (Intermined the Paula) Domain 1: Test Substance Metric 1: Test Substance Mune Comments Domain 1: Test Substance Metric 2: Test Substance Mune Low Domain 2: Test Design Metric 3: Test Substance Mune High Metric 6: Neadowcom High Substance Mune Metric 6: Neadowcom High Substance Mune Metric 6: Radowcom High Substance Mune Metric 6: Radowcom High The biological response: (Diff Oconentration) of the negative control group. Metric 6: Radowcom High Substance Mune Reporting onisions and appropriate cocurrent negative control group was ade quater. Domain 3: Exposure Charterization Keric 6: Radowcom Media Media Reporting onisions made assessing the experimental system of the substance Metric 6: Consistence y Of Exposure Of Exposure Of Exposure (Diff Oconentration) of the rest substan	Duration:	and Toxicolo Overall Dura	by $38(2):220-225$.	days	
Media Function Stars, Species, Age: Investmental: Stars, Species, Age: Investmental: Stars, Species, Age: Star	Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	dy authors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)
Taxa, species, Age: invertebrate; Atthropods; Aestna ap; Larvae Health Outcome: ADBK (biotransformation) Diethylhexyl phulalate (DFIP) Health Outcome and the field (Distransformation) Diethylhexyl phulalate (DFIP) Health Outcome and the field of the set	Media, Path:				
Tream Outcome: ADMic (notation) Dennical: Definition of the partial structure (DEHP) TIPEO ID: 700132 Domain 1: Test Substance Metric 1: Domain 1: Test Substance Metric 2: Domain 1: Test Substance Journey Ligh Domain 1: Test Substance Source Low Metric 2: Test Substance Pority Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Low Metric 7: Experimental System/Test Media Proparation Medium Metric 7: Experimental System/Test Media Proparation Medium Metric 7: Experimental System/Test Media Proparation Medium Metric 1: Number of Exposure Medium Metric 1: Constructorization Medium Metric 1: Number of Exposure Medium Metric 1: Number of Exposure Medium Metric 1: Number of Exposure Groups/ Metric 1: Metric 1: Metric 1: Number of Exposure Groups/ Metric 1: Test organism <td< th=""><th>Taxa, Species, Age:</th><th>Invertebrate;</th><th>(Arthropods; <i>Aeshna sp.</i>; Larvae</th><th></th><th></th></td<>	Taxa, Species, Age:	Invertebrate;	(Arthropods; <i>Aeshna sp.</i> ; Larvae		
HERO ID: 790132 Domain Metric Domain 1: Test Substance Kentric 1: Test Substance Clumity High Metric 2: Test Substance Surve Metric 3: Test Substance Purity Low The purity and/or grade of the test substance concurrent negative control group. Metric 4: Negative Controls Metric 5: Regative Controls Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Metric 7: Experimental System/Test Media Preparation Metric 9: Medianna Metric 10: Exposure Daration and Prequency Metric 11: Number of Test Substance Concentration Metric 12: Test for group Spacing of Exposure Daration and Prequency Metric 11: Number of Exposure Daration Metric 12: Test Organism Metric 13: Test Organism And and Prequency Metric 14: Acclimatization and Prequency Metric 15:	Chemical	Di-ethylbexy	ransformation) vl phthalate (DFHP)		
Domain 1: Test Substance Metric 1: Test Substance Identity High The chemical was identified by name. Domain 1: Test Substance Test Substance Source Low The source was not reported. Metric 2: Test Substance Purity Demine Purity The source was not reported. Domain 2: Test Design Metric 3: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Domain 3: Test Design Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Charteristics Keprimental System/Test Media Median Reporting omissions made assessing the experimental system and the methods for reported. Metric 6: Reporting Charteristics Mediance Reporting omissions made assessing the experimental system and the methods for reported. Metric 1: Sperimental System/Test Media Mediance Reporting omissions made assessing the experimental assessing the experimental system and the methods for reported concentrations were reported. Metric 1: Sperimental System/Test Media Mediance Reporting omissions made assessing the experimental aspect method set and the water concentration were reported. Metric 1: Number of Exposure Courses High Netric 1: Spating of Exposure Courses Metric 1: Number of Exposure Courses	HERO ID:	790132			
Domain 1: Test Substance Metric 1: Test Substance Identity High The chemical was identified by name. Metric 2: Test Substance Fouries Low The source was not reported. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Metric 5: Negative Control Response High The biological responses (DEIP concentration) of the negative control group. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test modia difficuit. Metric 11: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the water concentrations were reported. Metric 11: Number of Test Substance Medium Reporting omissions made assessing the experimental appropriate. Metric 12: The source Duration and Frequency High The duration of exposure and/or exposure reported. Metric 12: Test Organism Metric 13: Test Organism	Domain		Metric	Rating	Comments
Metric 1: Test Substance Identify High of the chemical was identified by name. Metric 2: Test Substance Querity Low The source was not reported. Domain 2: Test Substance Purity Low The source was not reported. Domain 2: Test Design Metric 4: Negative Controls High of the lobolical responses (DEHP concentration) of the negative control group. Metric 5: Negative Control Response High of the lobolical responses (DEHP concentration) of the negative control group. Domain 3: Exposure Characterization Response Metric 7: Metric 7: Experimental System/Test Media Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test modia difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test modia difficult. Metric 9: Measumement of Test Substance Medium Reporting omissions may have had a substantial impact on results. Administration Metric 10: Exposure Duration and Frequency High The doutnoin of exposure addres exposure concentrations were reported. Metric 12: Test Organism Low Reporting omissions prevented determ	Domain 1: Test Substan	ice		TT' 1	
Metric 2: Test Substance Source Low The source was not reported. Domain 2: Test Substance Purity Low The source was not reported. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the water concentration were reported. Metric 9: Consistency of Exposure Medium Reporting omissions may have had a substantial impact on results. Metric 10: Exposure Outarian and Frequency High The duration of exposure reported. Metric 11: Number of Exposure Groups/ Low Only two, nearly identical concentrations were reported. Metric 12: Test Organism Constitute exposure Groups/ Low Only two, nearly identical concentrations owere reported. Metric 12: Test Organism Constitute exposure Groups/ Low Constitute exposure concentration Domain 4: Test Organism Test Organism Characteristics Low <th></th> <td>Metric 1:</td> <td>Test Substance Identity</td> <td>High</td> <td>The chemical was identified by name.</td>		Metric 1:	Test Substance Identity	High	The chemical was identified by name.
Instance Instruct of the standard runty Dow The purty and/or grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using an appropriate concurrent negative control group. Metric 5: Negative Control Response High The biological responses (DEHP concentration) of the negative control group was adequate. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 9: Metsaurement of Test Substance Medium Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported. Metric 11: Number of Test Substance Medium Sediment concentrations were reported. Metric 12: Number of Test Substance Medium Sediment concentrations were reported. Metric 11: Number of Test Substance Medium Sediment concentrations were reported. Metric 12: Testing at or Below Solubility Limit Low Reporting omissions prevented determination of whether		Metric 2:	Test Substance Source	Low	The source was not reported.
Domain 2: Test Design Metric 4: Metric 5: Negative Controls High High High Study authors reported using an appropriate concurrent negative control group. High Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 9: Concentration Metric 9: Concentration and Frequency High The duration of exposure for uperstem tions were not reported. but the methods used and the water concentra- tions were not reported. Metric 10: Exposure Duration and Frequency Metric 11: High The duration of exposure indiver exposure for uperstem. Metric 12: Testing of Exposure Levels Metric 13: Low Reporting omissions mere actimations were reported. Domain 4: Test Organism Metric 13: Test Organism and Pretreatment Low There were significant concerns regarding the source of the test organisms. Metric 16: Adequacy of Test Conditions Low Reporting omissions were acclimatized to test temperatures. Domain 4: Test Organism Metric 13: Test Organism and Netric 14: Acclimat		Metric 5.	Test Substance Purity	LOW	The purity and/or grade of the test substance were not reported.
Metric 4: Negative Controls High Metric 5: Study authors reported using an appropriate concurrent negative control group. The biological responses (DEHP concentration) of the negative control group was ade- quate. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 9: Metric 8: Consistency of Exposure Metric 9: Medium Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported. Metric 10: Exposure Duration and Frequency High High The dwaling of exposure frequency were reported and appropriate. Metric 12: Resposure Groups/ Spacing of Exposure Groups/ Metric 12: Low Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Domain 4: Test Organism Metric 15: Test Organisms and Replicates per Group Low Reporting omissions prevented determination to exposure chambers were similar in concentration but not really reps. Considered rating this metri	Domain 2: Test Design				
Metric 5: Negative Control Response High quark The biological responses (DEHP concentration) of the negative control group was adequate. Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions may have had a substantial impact on results. Administration Administration Medium Sediment concentration of exposure forquency were reported, but the methods used and the water concentrations were not reported. Metric 10: Exposure Duration and Frequency High The duration of exposure forquency were reported. Metric 11: Number of Exposure Croups/ Low Only two, nearly identical concentrations were reported. Spacing of Exposure Levels Test organism Low Reporting omissions prevented determination of whether exposure concentration exceled the water solubility limit. Domain 4: Test Organism Conclinato		Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions may have had a substantial impact on results. Administration Metric 9: Measurement of Test Substance Medium Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported. Metric 10: Exposure Duration and Frequency High The duration of exposure frequency were reported and appropriate. Metric 12: Testing at or Below Solubility Limit Low Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism characteristics Low There were significant concerns regarding the source of the test organisms. Metric 15: Number of Organisms and Replicates per Group Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 5:	Negative Control Response	High	The biological responses (DEHP concentration) of the negative control group was ade- quate.
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Medium Reporting omissions made assessing the experimental system and the methods for preparation Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 9: Metric 9: Medium Reporting omissions may have had a substantial impact on results. Administration Medium Sediment concentrations were reported, but the methods used and the water concentrations were reported. Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate. Metric 11: Number of Exposure Groups/ Low Only two, nearly identical concentrations were reported. Spacing of Exposure Levels Metric 13: Test Organism Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 13: Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Metric 15: Number of Organisms and Replic		Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Metric 7: Experimental System/Test Media Preparation Medium Preparation Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 8: Consistency of Exposure Medium Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult. Metric 9: Metaurement of Test Substance Medium Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported. Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate. Metric 12: Number of Exposure Coroups/ Low Only two, nearly identical concentrations were reported. Spacing of Exposure Levels Metric 13: Test Organism Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 15: Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low	Domain 3: Exposure Ch	naracterization			
Metric 8: Consistency of Exposure Medium Reporting omissions may have had a substantial impact on results. Metric 9: Administration Metric 0: Exposure Duration and Frequency Medium Sediment concentrations were reported, but the methods used and the water concentrations were not reported. Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate. Metric 11: Number of Exposure Groups/ Low Only two, nearly identical concentrations were reported. Spacing of Exposure Levels Metric 12: Test Organism Characteristics Low Metric 14: Acclimatization and Pretreatment Medium There were significant concerns regarding the source of the test organisms. Metric 15: Test Organism and Reprictions Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 7:	Experimental System/Test Media Preparation	Medium	Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult.
Metric 9: Measurement of Test Substance Concentration Medium Medium Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported. Metric 10: Exposure Duration and Frequency High The duration of exposure frequency ever reported and appropriate. Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Low Only two, nearly identical concentrations were reported. Metric 12: Test organism Low Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Conditions Metric 15: Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 8:	Consistency of Exposure	Medium	Reporting omissions may have had a substantial impact on results.
Metric 10: Exposure Duration and Frequency Metric 11: High Number of Exposure Groups/ Spacing of Exposure Groups/ Low The duration of exposure and/or exposure frequency were reported and appropriate. Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Low Only two, nearly identical concentrations were reported. Metric 12: Testing at or Below Solubility Limit Low Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Conditions Metric 15: Number of Organisms and Replicates per Group Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 9:	Measurement of Test Substance Concentration	Medium	Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported.
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Low Only two, nearly identical concentrations were reported. Metric 12: Testing at or Below Solubility Limit Low Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Conditions Conditions Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate.
Spacing of Exposure Levels Testing at or Below Solubility Limit Low Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. The test organisms were acclimatized to test temperatures. Conditions Metric 15: Conditions Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 11:	Number of Exposure Groups/	Low	Only two, nearly identical concentrations were reported.
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Conditions Conditions Number of Organisms and Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Metric 15: Conditions Number of Organisms and Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.					
Metric 15. Test Organism Characteristics Low There were significant concerns regarding the source of the test organisms. Metric 14: Acclimatization and Pretreatment Medium The test organisms were acclimatized to test temperatures. Metric 15: Number of Organisms and Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.	Domain 4: Test Organis	Matric 12:	Test Organism Characteristics	Low	There were significant concerns recording the source of the test erections
Metric 14: Attentinatization and Prefetation Metric 16: Attentinatization and Prefetation Metric 15: Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 14:	Acclimatization and Pretreatment	Low Medium	There were significant concerns regarding the source of the test organisms.
Metric 15: Number of Organisms and Replicates per Group Low Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Weute 14.	Conditions	Weddulli	The test organisms were accumatized to test temperatures.
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.		Metric 15:	Number of Organisms and Replicates per Group	Low	Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric unacceptable.
Metric 16: Adequacy of Test Conditions Low Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.	Domain 5: Outcom- A-				
Continued on pext page	Domain 5: Outcome As	Metric 16	A dequacy of Test Conditions	Low	Reporting omissions led to uncertainty regarding whether the organism environmental
Continued on next name		wieute 10:	Auquacy of rest conditions	LOW	conditions were conducive to the maintenance of health.
continued on next page			(Continued on next page	

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Diethylhexyl Phthalate

HERO ID: 790132 Table: 1 of 2

		col	ntinued from previous	page			
Study Citation:	Woin, P., La and Toxicolo	Woin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination and Toxicology 38(2):220-225.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Aeshna sp.; Larvae					
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	790132						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not addressed.			
	Metric 18:	Consistency of Outcome	Low	Details of the outcome assessment protocol were not reported.			
		Assessment					
Domain 6: Confounding	/ Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not performed.			
	Metric 22:	Reporting of Data	Low	It was unclear which sediment concentration was system 1 or system 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Woin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination and Toxicology 38(2):220-225. Overall Duration: > 21 days: Exposure Duration: > 21 days				
Exposure Route, Media, Path:	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i.e., cl	hemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Invertebrate;	Arthropods; Aeshna sp.; Larvae			
Health Outcome:	Behavioral				
Chemical: HERO ID:	Di-ethylhexy 790132	yl phthalate (DEHP)			
Domain	190132	Metric	Rating	Comments	
Domain 1: Test Substan	ce	monie	Ruting	Comments	
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.	
	Metric 2:	Test Substance Source	Low	The source was not reported.	
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	High	The biological responses (feeding behavior) of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Reporting omissions made assessing the experimental system and the methods for preparation of the test media difficult.	
	Metric 8:	Consistency of Exposure	Low	Reporting omissions may have had a substantial impact on results.	
	Metric 9:	Administration Measurement of Test Substance	Medium	Sediment concentrations were reported, but the methods used and the water concentra- tions were not reported	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate.	
	Metric 11:	Number of Exposure Groups/	Low	Only two, nearly identical concentrations were reported.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented the determination of whether the exposure concentra-	
				tions exceeded the water solubility limit.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Low	There were significant concerns regarding the source of the test organisms.	
	Metric 14:	Acclimatization and Pretreatment	Medium	The test organisms were acclimatized to test temperatures.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	Replicates were not used. Two exposure chambers were similar in concentration but not really reps. Considered rating this metric as unacceptable.	
		1 L ab		. .	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting omissions led to uncertainty regarding whether the organism environmental conditions were conducive to the maintenance of health.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.	
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
		Conti	nued on next pa	ge	

HERO ID: 790132 Table: 2 of 2

Study Citation:	Woin, P., La	Voin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination				
D (1	and Toxicolo	ogy 38(2):220-225.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	y authors (1.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	T					
Taxa, Species, Age:	Invertebrate;	abaviaral				
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	/90132					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Low Medium	The study did not provide enough information to allow a comparison of environmental conditions. There was no information in the study to suggest differences among groups		
	100010 20.		meanam	There was no mitorination in the study to suggest universes among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	It was unclear which sediment concentration was system 1 or system 2.		
	16	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	Metric 23:	Explanation of Onexpected Outcomes		There were no unexpected outcomes.		

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Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973). To	oxicity, residue dynamics	s, and reproductive effects of phthalate esters in aquatic invertebrates.
	Environmen	tal Research 6(1):84-90.	21.1	
Duration: Exposure Doute	A quatia (from	ation: 11 - 21 days; Exposure Duration: 11 -	21 days	toract in avacuura water, but unable to determine event untake route)
Media Path	Aquatic (free	sinwater), water, not determined by study au	iulois (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake foute)
Taxa. Species. Age:	Invertebrate:	Arthropods: Asellus breviicaudus: Not App	licable (e.g., fungi or alga	e studies) or Not Reported
Health Outcome:	ADME (biot	transformation)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	1334646			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce	— — — — — — — — — —		
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
ľ	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Low	Few details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Low	Only one treatment was reported.
	14.1.10	Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Low	The number of test replicates was not reported.
		Replicates per Group		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently detailed to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.
		С	ontinued on next page .	

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Diethylhexyl Phthalate

HERO ID: 1334646 Table: 1 of 1

		com	ntinued from previous	s page		
Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973). Tox	cicity, residue dynami	cs, and reproductive effects of phthalate esters in aquatic invertebrates.		
	Environmen	tal Research 6(1):84-90.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 - 2	1 days			
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Asellus breviicaudus; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	ADME (biot	ADME (biotransformation)				
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1334646					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for outcome assessment were		
		Assessment		provided.		
Domain 6: Confounding	o / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not performed.		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for the sampling period.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quali	ty Detern	nination	Uninformativ	7 e		

Study Citation: Duration: Exposure Route, Media, Path:	Cruciani, V., Brachionus c Overall Dura Aquatic (fres	Cruciani, V., Iovine, C., Thomé, J. P., Joaquim-Justo, C. (2015). Impact of three phthalate esters on the sexual reproduction of the Monogonont rotifer, Brachionus calyciflorus. Ecotoxicology 25(1):192-200. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate;	Other Invertebrate (e.g., sea urchins, ciliates	s, rotifers); Brac	hionus calyciflorus; Pallas; Larvae		
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	3070931					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The test substance was identified only by the chemical name. No other information was provided.		
	Metric 2:	Test Substance Source	Low	The test substance was obtained from Sigma Aldrich, Germany, but the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure). Both water and solvent controls were used.		
	Metric 5:	Negative Control Response	High	The biological responses of the controls were reported and were adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of test substance before and during the expo- sure. Concentrations of the test substance were not measured during the study.		
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the initial exposure administration were reported, but details of the exposure administration past 48 hours were not clearly reported. It was reported that at 48 hours, rotifers were transferred to new test media, and at 48, 72 and 96 hours, tubes were emp- tied into a glass petri dish for counting males and females. No details were given on how the experiment was continued until 96 hours.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcomes of interest.		
	Metric 11:	Number of Exposure Groups/	High	There were 4 exposure groups for DEHP, and 5 replicates were run for each concentra-		
		Spacing of Exposure Levels		tion.		
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate (i.e., no effects on biological responses were observed in the solvent control and no interactions were expected between the solvent and test substance).		
Domain 4. Test Organis	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
		Contin	ued on next pa	ge		

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Environmental Hazard Evaluation

HERO ID: 3070931 Table: 1 of 1

		contin	ued from previ	ous page
Study Citation:	Cruciani, V. Brachionus	, Iovine, C., Thomé, J. P., Joaquim-Justo, C calyciflorus. Ecotoxicology 25(1):192-200.	C. (2015). Impac	t of three phthalate esters on the sexual reproduction of the Monogonont rotifer
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study at	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Invertebrate	; Other Invertebrate (e.g., sea urchins, ciliate	s, rotifers); Brac	chionus calyciflorus; Pallas; Larvae
Health Outcome:	Reproductiv	re/Teratogenic		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	3070931			
Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characteriz toxicological effects.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, and food were conducive to the mainte- nance of health. It was reported that the population growth rate at 48 h in the control w greater than 0.7, which is indicative of healthy conditions.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. Methodology consisted of counting non-ovigerous females, amictic ovigerous females, mictic ovigerous females, fertilized females, fertilized eggs carried per fertilized female, and detached fertilized eggs. In the paper and in the cited reference (Preston et al. 2000), it was reported that test tube contents were emptied into a petri dish at 48 and 72 hours, but there were no details given regarding the methods for continuing the experiment until 96 hours.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Outcomes were assessed at 48, 72 and 96 hours in treatment groups and controls.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.
Domain 7: Data Present	tation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	DEHP expos	sure (0.05 to 2mg/L) caused no significant et	ffect on populati	on growth rate, mixis rate, fertilization rate, or resting egg production in rotifers.
		•	N. 7. 14	

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route,	Zhao, L. L., Xi, Y. L., Huang, L., Zha, C. W. (2009). Effects of three phthalate esters on the life-table demography of freshwater rotifer Brachionus calyciflorus Pallas. Aquatic Ecology 43(2):395-402. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path: Taxa, Species, Age: Health Outcome:	Invertebrate; Mortality	Arthropods; Brachionus calyciflorus; Juveni	le			
Chemical: HERO ID:	Di-ethylhexy 1336226	l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The source of the test substance was Sigma Aldrich (Germany). The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as $>=97\%$.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were inadequate for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolu- tion.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	Eggs were collected in the sediments of Lake Jinghu and cultured in the lab. There are minor reservations regarding the source of test organisms that are unlikely to have a substantial impact on results.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

		contin	ued from previo	nus page				
Study Citation:	Zhao, L. L.,	Zhao, L. L., Xi, Y. L., Huang, L., Zha, C. W. (2009). Effects of three phthalate esters on the life-table demography of freshwater rotifer Brachionus calvaitlearus Pallae. Aquatic Ecology 43(2):305-402						
Duration:	Overall Dura	Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days						
Exposure Route,	Aquatic (free	Aduatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Brachionus calyciflorus; Juven	ile					
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	1336226	-						
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None							
Overall Qualit	ty Detern	nination	Medium					

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Study Citation: Duration: Exposure Route, Media Path:	Zhao, L. L., Xi, Y. L., Huang, L., Zha, C. W. (2009). Effects of three phthalate esters on the life-table demography of freshwater rotifer Brachionus calyciflorus Pallas. Aquatic Ecology 43(2):395-402. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 1336226						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High High	The chemical was identified by name. The source of the test substance was Sigma Aldrich (Germany). The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as $>=97\%$.			
Domain 2: Tast Dasign							
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Demain 2: Erreener Ch							
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Hich				
	Metric 7.	Preparation	nigii	scribed in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were inadequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolu- tion.			
Domain 4. Test Organis	-						
	Metric 13:	Test Organism Characteristics	Medium	Eggs were collected in the field and cultured in the lab. There are minor reservations regarding the source of test organisms that are unlikely to have a substantial impact on results.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.			
Continued on next page							

Diethylhexyl Phthalate

Metric 22:

Metric 23:

None

Overall Quality Determination

Additional Comments:

Reporting of Data

Explanation of Unexpected Outcomes

Data for exposure-related findings were presented for each treatment and control group.

There were no unexpected outcomes.

... continued from previous page **Study Citation:** Zhao, L. L., Xi, Y. L., Huang, L., Zha, C. W. (2009). Effects of three phthalate esters on the life-table demography of freshwater rotifer Brachionus calyciflorus Pallas. Aquatic Ecology 43(2):395-402. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Exposure Route, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Brachionus calyciflorus; Juvenile **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) 1336226 HERO ID: Domain Metric Rating Comments Outcome Assessment Methodology High Metric 17: The outcome assessment methodology reported the intended outcome of interest. Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures High Metric 20: Outcomes Unrelated to Exposure There were no differences among groups. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were adequately described.

High

High

Medium

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Study Citation:	Streufert, J.	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of						
Duration:	Overall Dur	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) A quetic (freeburgter); Weter Net determined by study authors (i.e., chemical of interact in exposure water, but upplie to determine exact uptalic route).						
Media. Path:	Aquatic (neshwater), water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Chironomus plumosus; Larvae						
Health Outcome:	Immobilizat	ion						
Chemical:	Di-ethylhex	yl phthalate (DEHP)						
HERO ID:	813673							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce Matria 1	Trat Cubatan an Idantita	I					
	Metric 1: Metric 2:	Test Substance Identity	Low	The DEHP was identified by name only. The source of the DEHP was reported to be Mansanto Chamical Supply in St. Louis				
	Wieuric 2.	Test Substance Source	Low	MO. It was not reported if the DEHP was analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.				
Domain 2: Test Design	Matria 4.	Nagativa Controla	Iliah					
	Metric 4:	Negative Controls	nigii	used.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported. Only EC50 values were reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.				
Domain 3: Exposure Ch	Matria 7:	Experimental System/Test Media	Low	Little information was provided on the propagation of the test modio				
	Metric 7.	Preparation	LOW	Little information was provided on the preparation of the test media.				
	Metric 8:	Consistency of Exposure Administration	High	Study authors cited the Committee on Methods of Toxicity Tests with Aquatic Organ- isms, 1975 for the methods used in the acute toxicity tests.				
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported.				
	Metric 10:	Exposure Duration and Frequency	Medium	The study duration was reported to be 48h. 96h tests are typical for midge acute toxicity tests. The test duration did not appear to have a significant effect on the outcome.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The study authors did not report the number of exposure groups or the spacing of the groups for the acute tests.				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under 0.1mL/L in all test concentrations and controls.				
Domain 4: Test Organis	m Matria 12:	Test Organism Characteristics	Uich	The test argonisms were obtained from on in house culture and were the encountries				
	wieuric 15:	rest Organism Characteristics	nigii	for the study.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report if the organisms were acclimated or needed to be acclimated to test conditions.				
Continued on next page								

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Diethylhexyl Phthalate

		con	tinued from previou	s page				
Study Citation:	Streufert, J.	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route.	Aquatic (fre	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	1		× ,					
Taxa, Species, Age:	Invertebrate	; Arthropods; Chironomus plumosus; Larvae						
Health Outcome:	Immobilizat	ion						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	813673							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and	Low	The number of test organisms per test chamber and the number of replicates was not				
		Replicates per Group		reported, though this may have been included in the citation for methodology.				
Domain 5: Outcome As	ssessment							
	Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. It was not reported if the organisms were fed or what the biomass per test chamber was.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-immobilization was reported in EC50 values.				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol were not reported. Organisms were assessed for immobilization, but it was not clear how this was done.				
Domain 6: Confoundin	g / Variable Co	ntrol	τ					
	Metric 19:	Design and Procedures	Low	conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Presen	tation and Anal	lysis						
	Metric 21:	Statistical Methods	High	Methods described by Litchfield and Wilcoxon 1949 were used to determine EC50 values.				
	Metric 22:	Reporting of Data	Low	Only EC50 values were reported.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 2.				
Additional Comments:	This portion selected as t	of the evaluation was on the acute toxicity of L he outcome for the evaluation. The study received	DEHP on C. pulmosus ved an unacceptable r	. The study reported assessing immobilization as the outcome, so that was anking due to the lack of reporting on exposure groups and spacing.				

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 813673 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media, Path:	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus plumosus; Larvae				
Health Outcome:	ADME (biot	ransformation)				
Chemical: HFRO ID:	Di-ethylhexy	/I phthalate (DEHP)				
Domain	015075	Metric	Dating	Comments		
Domain 1: Test Substan	ce	metre	Kaung	Comments		
Domain 1. 10st Substan	Metric 1:	Test Substance Identity	Low	The DEHP was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source of the 14C labelled DEHP was reported to be Pathfinders Laboratories Inc. in St. Louis, MO. It was not reported if the DEHP was analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Uninformative	This portion of the study did not report the use of a concurrent negative control.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported as there did not appear to be a negative control.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.		
Domain 2: Exposure Ch	aractarization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test media and concentrations. A diluter system was reported to be used for this portion of the study.		
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. A diluter system was used to administer the test media, but the test chambers were not described. Test volumes were also not described.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported. Other sources were cited.		
	Metric 10:	Exposure Duration and Frequency	High	It was reported there was a 4 day exposure with a 5 day period in which midges were in DEHP-free water.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level for this portion of the study, as the goal was not to have a dose response, but to observe any accumulation of DEHP in midge tissue.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from an in-house culture and the age of the organisms was appropriate.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report if the organisms were acclimated or needed to be acclimated to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms for this portion of the study was not reported, nor was the number of replicates.		
Domain 5: Outcome As	sessment					

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 813673 Table: 2 of 2

		com	tinued from previou	s page				
Study Citation:	Streufert, J. the Missouri	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40.						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus plumosus; Larvae							
Health Outcome:	ADME (biot	transformation)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	813673							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. It was not reported if the midges were fed or what the biomass loading was.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DEHP tissue accumulation–ADME.				
	Metric 18:	Consistency of Outcome	Low	It was unclear how the DEHP in the midge tissue was determined.				
		Assessment						
Domain 6: Confoundir	ng / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7. Data Preser	station and Anal	veie						
Domain 7. Data Meser	Metric 21.	Statistical Methods	N/A	There was only 1 treatment level and no control, so there was nothing to compare				
	Metric 22:	Reporting of Data	Low	The exposure response was reported in Figure 1 but there was not a control response				
	Methe 22.	Reporting of Dutu	Low	reported.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was reported in Figure 1. The study authors did not report any unexpected outcomes.				
Additional Comments:	This portion	of the evaluation was on the accumulation of	14C labelled DEHP i	n midge tissue. ADME was selected as the outcome of interest. This part				

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae						
Taxa, Species, Age:							
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	1332972						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		-				
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.			
	Metric 2:	Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.			
	Metric 3:	Test Substance Purity	Low	No purity was reported.			
Domain 2. Test Design							
	Metric 4:	Negative Controls	Low	Ethanol was listed for solvent controls, however, authors report using ethanol concentra- tions at higher levels than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of the compounds.			
	Metric 5:	Negative Control Response	Low	Control responses were not reported for acute toxicity bioassays.			
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal with mortality as- sessed at 24 and 48 hours.			
	Metric 8:	Consistency of Exposure	High	The exposure administration appeared consistent among treatments and control.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	The acute bioassay concentrations were not analyzed, and LC50 concentrations were reported as nominal.			
	Metric 10:	Exposure Duration and Frequency	High	The duration (48 hr) is appropriate.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	None of the treatment concentrations for the acute bioassays were reported. Range find- ing tests were not described.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Authors intentionally exceeded solubility concentrations for the acute bioassays. The range of concentrations was not reported, but LC50 values were above the solubility reported for this compound in the Final Scope (0.27 mg/L).			
Domain 4: Test Organics	m						
	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.			
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The authors listed 10 individuals per treatment concentrations, but they did not report the level of replication for each treatment.			

Domain 5: Outcome Assessment

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

May 2025 Environmental Hazard Evaluation

		contir	nued from previo	us page			
Study Citation: Duration: Exposure Route,	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae Mortality Di-ethylhexyl phthalate (DEHP) 1332972						
Domain		Metric	Rating	Comments			
	Metric 16: Metric 17:	Adequacy of Test Conditions Outcome Assessment Methodology	Medium High	DO, temperature, and photoperiod were reported for the acute bioassays. The authors had a very detailed list of multiple criteria to determine mortality from the bioassays.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.			
Domain 6: Confounding	/ Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmen- tal conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21: Metric 22:	Statistical Methods Reporting of Data	High Medium	Authors used Litchfield and Wilcoxon method for LC50 estimation. LC50 and 95% confidence intervals are reported, but results from each concentration are not available.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Quality Determination			Medium				

Study Citation: Duration: Exposure Route, Media Path:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome:	Invertebrate Mortality	Arthropods; <i>Chironomus plumosus</i> ; Larvae				
HERO ID:	1332972	(DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.		
	Metric 2:	Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.		
	Metric 3:	Test Substance Purity	Low	No purity was reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	Ethanol was listed for solvent controls, however, authors report using ethanol concentra- tions at higher levels than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of the compounds.		
	Metric 5:	Negative Control Response	Low	Control responses were not reported for the acute toxicity bioassays.		
	Metric 6:	Randomized Allocation	Low	It was not reported how animals were allocated to treatment concentrations.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal with mortality as- sessed at 24 and 48 hours.		
	Metric 8:	Consistency of Exposure	High	Exposure administration appeared consistent among treatments and control.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	The acute bioassay concentrations were not analyzed, and LC50 concentrations are reported as nominal.		
	Metric 10:	Exposure Duration and Frequency	High	The duration (48 hr) was appropriate.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	None of the treatment concentrations for the acute bioassays were reported. Range find- ing tests were not described.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Authors intentionally exceeded solubility concentrations for the acute bioassays. The range of concentrations was not reported, but LC50 values are above the solubility reported for this compound in the Final Scope (0.27 mg/L).		
Domain 4. Test Organia						
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.		
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The authors listed 10 individuals per treatment concentration, but they did not report the level of replication for each treatment.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the acute bioassays.		
Continued on next page						

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Diethylhexyl Phthalate

		contin	ued from previ	ous page		
Study Citation: Duration:	Streufort, J. Overall Dura	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	; Arthropods; Chironomus plumosus; Larvae	•			
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1332972					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The authors had a very detailed list of multiple criteria to determine mortality from the bioassays.		
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.		
Domain 6: Confoundin	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmen- tal conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health out- comes.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Authors used Litchfield and Wilcoxon method for LC50 estimation.		
	Metric 22:	Reporting of Data	Medium	LC 50 and 95% confidence intervals are reported, but results from each concentration are not available.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973). T	Coxicity, residue dynamics	s, and reproductive effects of phthalate esters in aquatic invertebrates.			
Duration: Exposure Route, Media, Path:	Environment Overall Dura Aquatic (fres	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	nvertebrate; Arthropods; Chironomus plumosus; Larvae					
Health Outcome:	ADME (biot	ADME (biotransformation)					
HERO ID:	1334646	(DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Low	Few details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Low	Only one treatment was reported.			
	Matria 12.	Spacing of Exposure Levels	Iliah				
	Metric 12:	Testing at of Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.			
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.			
			Continued on next page .				

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Diethylhexyl Phthalate

HERO ID: 1334646 Table: 1 of 1

		0	continued from previous pa	ge		
Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973).	Foxicity, residue dynamics,	and reproductive effects of phthalate esters in aquatic invertebrates.		
	Environmen	tal Research 6(1):84-90.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus plumosus; Larvae	e			
Health Outcome:	ADME (biot	transformation)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1334646					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for outcome assessment were		
		Assessment		provided.		
Domain 6: Confounding	/ Variable Co	ntrol				
Domain 0. Comounding	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmenta		
		Design and Procedures		conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not performed.		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for the sampling period.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Uninformative			

Study Citation: Duration:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days						
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	Laurante hantes Anthere and a Chinese and the second second second second second second second second second se						
Health Outcome:	ADME (biot	ransformation)	ac				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1332972						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.			
	Metric 2:	Test Substance Source	Low	The source of radio labeled DEHP was listed as Pathfinder laboratories, but it was not analytically verified.			
	Metric 3:	Test Substance Purity	Low	No purity was reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	N/A	For this ADME study, the authors do not use a control.			
	Metric 5:	Negative Control Response	N/A	No control treatment within this ADME study.			
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The larval uptake assays were conducted as static non-renewal.			
	Metric 8:	Consistency of Exposure	High	The exposure administration appeared consistent among treatments and control.			
	Metric 9:	Measurement of Test Substance Concentration	High	GC was used to verify concentrations throughout the uptake study within the water, organisms, and substrate.			
	Metric 10:	Exposure Duration and Frequency	High	120 hours is appropriate for this bioassay.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The initial concentrations were chosen from the labs previous work on similar chemi- cals.			
	Metric 12:	Testing at or Below Solubility Limit	High	The concentrations within this ADME study were at the reported solubility limit for DEHP (0.27 mg/l).			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.			
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the uptake and elimination study (8 days).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Samples sizes and procedures were appropriate for this ADME study.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of fest Conditions	Medium	water quality parameters were monitored for other studies within the manuscript but not reported for this experiment.			
	Continued on next page						

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days **Duration:** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae **Health Outcome:** ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1332972 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology High The calculations of a quench curve for tissues was reported on page 30/62. Analytical methods for concentration determination of tissue, water, and substrate were also reported throughout time. Metric 18: Consistency of Outcome High The outcomes appear to be reported consistently. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: High Nothing was reported to indicate differences among treatment groups with environmen-Design and Procedures tal conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information to suggest differences among groups related to health outcomes. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High These data were plotted (page 47/62), and elimination curves were created and presented with R values. Metric 22: Reporting of Data High Data at all time points are presented in tables 15 and 16. Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported. Additional Comments: None

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate: Arthropods: <i>Chironomus plumosus</i> : Larvae						
Health Outcome:	ADME (biot	ADME (biotransformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1332972	1332972					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.			
	Metric 2:	Test Substance Source	Low	The source of the radio labeled DEHP was listed as Pathfinder laboratories, but it was not analytically verified.			
	Metric 3:	Test Substance Purity	Low	No purity was reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	N/A	For this ADME study, the authors do not use a control.			
	Metric 5:	Negative Control Response	N/A	There was no control treatment within this ADME study.			
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The larval uptake assays were conducted as static non-renewal.			
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and controls.			
	Metric 9:	Measurement of Test Substance Concentration	High	GC was used to verify concentrations throughout the uptake study within the water, organisms, and substrate.			
	Metric 10:	Exposure Duration and Frequency	High	120 hours is appropriate for this bioassay.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The initial concentrations were chosen from the labs previous work on similar chemi- cals.			
	Metric 12:	Testing at or Below Solubility Limit	High	The concentrations within this ADME study were at the reported solubility limit for DEHP (0.27 mg/l).			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.			
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the uptake and elimination study (8 days).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Samples sizes and procedures were appropriate for this ADME study.			
Domain 5: Outcome As	sessment						
20man 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Water quality parameters were monitored for other studies within the manuscript but not reported for this experiment.			
Continued on next page							

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days **Duration:** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae **Health Outcome:** ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1332972 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology High The calculations of a quench curve for tissues was reported on page 30/62. Analytical methods for concentration determination of tissue, water, and substrate were also reported throughout time. Metric 18: Consistency of Outcome High The outcomes appear to be reported consistently. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: High Nothing was reported to indicate differences among treatment groups with environmen-Design and Procedures tal conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information to suggest differences among groups related to health outcomes. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High These data were plotted (page 47/62), and elimination curves were created and presented with R values. Metric 22: Reporting of Data High Data at all time points are presented in tables 15 and 16. Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported. Additional Comments: None

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A	A. (1982). Significance of interfaces in the dis	stribution and metabolism	n of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.			
Duration	Environmen	tal Pollution 27(4):263-274.	lava				
Duration: Exposure Route.	Aquatic (fre	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media. Path:	Aquatic (IIC	sinwater), water, not determined by study au	ulors (i.e., ellennear of in	terest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Chironomus plumosus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (bio	ADME (biotransformation)					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	59542						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were reported.			
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.			
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.			
Domain 2: Test Design							
6	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.			
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.			
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media	Medium	Biomass loading and placement of organisms in a 20L were tank not reported.			
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding to the system.			
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).			
	Metric 10:	Exposure Duration and Frequency	High	A 27-day exposure was sufficient.			
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L, which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.			
Demain 4. Test O							
Domain 4: Test Organisi	II Matric 12:	Test Organism Characteristics	Low	Organisms ware collected in the field. Age and say ware not provided in the study			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment			
	Meule 14.	Conditions	LOW	The study and not report acclimation of protectiment.			
	Metric 15:	Number of Organisms and	Low	No replicates were reported.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page		
Study Citation:	Sodergren, A Environment	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus plumosus; Not Appl	icable (e.g., fungi or	algae studies) or Not Reported		
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	59542					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of feed for fish were not well described.		
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as in water and in sediment were described, but details of organism preparation for extraction was not described (euthanasia, if applicable, or harvesting of plant material).		
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.		
Domain 6: Confounding	/ Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP uptake by each organism.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.		
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.		
Additional Comments:	This study c occurred. Or may not be a DEHP + met	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams. Collection of water and sediment for the mesocosm also occurred. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites. The metabolites were phthalic acid and phthalic anhydride.				

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of						
Duration:	the Missouri Overall Dura	i Academy of Science 14:33-40. ation: > 21 days: Exposure Duration: > 21 d	davs				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	; Arthropods; Chironomus plumosus; Larvae					
Health Outcome:	Reproductiv	e/Teratogenic					
Unemical:	Di-ethylnexy	DI-entymexyl phinalate (DEHP) 813673					
	813073						
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	Metric 1:	Tast Substance Identity	Low	The DEUD was identified by nome only			
	Metric 2.	Test Substance Source	Low	The source of the DEHP was reported to be Monsanto Chemical Supply in St. Louis			
	Wieule 2.	Test Bubstance Bource	Low	MO. It was not reported if the DEHP was analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The study reported the use of a concurrent negative control in which the solvent was used.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and was reported in Table 4.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.			
Domain 5: Exposure Cr	Matric 7:	Experimental System/Test Media	Low	Little information was provided on the propagation of the test made and concentrations			
	Wieuric 7.	Preparation	Low	A diluter system was reported to be used for the chronic test.			
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. A diluter system was used to administer the test media, but the test chambers were not described. Test volumes were also not described. It is unclear at what point the F0 adults were mated for obtain- ing egg masses. It is unclear if the embryos were exposed or just monitored for hatch.			
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported. Other sources were cited.			
	Metric 10:	Exposure Duration and Frequency	Uninformative	The duration for this portion of the chronic test was not reported. No more adults were reported to be emerging after 35 days, but it is unclear when the exposed F0 generation were mated or for how long the egg masses were exposed.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 3 reported exposure levels plus a control. More exposure levels or different spacing of the exposure levels may have yielded more in depth results.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under 0.1mL/L in all test concentrations and controls.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from an in-house culture and were the appropriate age for the study.			
		С	Continued on next page .				
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Diethylhexyl Phthalate

		con	tinued from previous	5 page		
Study Citation:	Streufert, J. I the Missouri	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 day	ys			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study auth	iors (i.e., chemical of i	nterest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus piumosus; Larvae Reproductive/Teratogenic					
Chamical	Reproductive/ Teratogenic Di-ethylbeyyl phthalate (DEHP)					
HFRO ID.	813673	n philalate (DEHF)				
	813073					
Domain	N. 4 . 14	Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report if the organisms were acclimated or needed to be acclimated to test conditions.		
	Metric 15:	Number of Organisms and	Low	Study authors reported using 100 organisms for the chronic test, but it was unclear how		
		Replicates per Group		many replicates there were and how many organisms were in each exposure group.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. Organisms were fed 0.12g of dog candy daily until pupation. It was not reported how many organisms were in each test chamber.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–egg mass production, number of eggs per mass, and hatchability of the eggs.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the outcome assessment were confusing and limited. It is unclear when the egg masses were counted and when the eggs were counted.		
Domain 6: Confounding	g / Variable Coi	ntrol	T			
	Metric 19:	Confounding variables in Test	Low	I he study did not provide enough information to allow a comparison of environmental conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	veis				
	Metric 21.	Statistical Methods	Low	Statistical analysis was performed for this portion of the study, but it was not described		
	Metric 22:	Reporting of Data	High	Control and exposure response data was reported in Table 4 and was adequate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This portion of the evaluation was on the chronic toxicity of DEHP on midge reproduction. The number of egg masses produced and the number of eggs per egg mass were reported, so the reproductive outcome was selected. This part received an unacceptable rating due to the lack of information regarding the duration. It is unclear when the F0 adults were mated and when the eggs were counted. It appears as though the egg masses were then kept at the same exposure level as the F0 generation, so mortality we be the outcome of interest for the hatchability portion of the study.					
Overall Qualit	ty Detern	nination	Uninformativ	<i>r</i> e		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of						
	the Missouri	the Missouri Academy of Science 14:33-40.					
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Arthropods; Chironomus plumosus; Larvae	2				
Health Outcome:	Developmer	nt/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	813673						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be Monsanto Chemical Supply in St. Louis.			
				MO. It was not reported if the DEHP was analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.			
		5					
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	The study reported the use of a concurrent negative control in which the solvent was			
		e	U	used.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and was reported in Table			
			-	3.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.			
Domain 3: Exposure Ch	aracterization		.				
	Metric /:	Experimental System/Test Media	Low	Little information was provided on the preparation of the test media and concentrations.			
		Preparation	Ŧ	A diluter system was reported to be used for the chronic test.			
	Metric 8:	Consistency of Exposure	Low	Details regarding the exposure administration were limited. A diluter system was used			
		Administration		to administer the test media, but the test chambers were not described. Test volumes			
	Matria 0.	Massurement of Test Substance	Madium	Were also not described.			
	Metric 9.	Concentration	wiedłum	methods used were not reported. Other sources were cited			
	Metric 10.	Exposure Duration and Frequency	Low	The duration was reported to be 2 days post when the last cast off pupal skin was found			
	Methe 10.	Exposure Duration and Frequency	Low	According to Table 3 this was 35 days though it was not explicitly stated anywhere in			
				the article.			
	Metric 11:	Number of Exposure Groups/	Medium	There were 3 reported exposure levels plus a control. More exposure levels or different			
		Spacing of Exposure Levels		spacing of the exposure levels may have yielded more in depth results.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under			
				0.1mL/L in all test concentrations and controls.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from an in-house culture and were the appropriate age for the study.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report if the organisms were acclimated or needed to be acclimated to			
		Conditions		test conditions.			
	Metric 15:	Number of Organisms and	Low	Study authors reported using 100 organisms for the chronic test, but it was unclear how			
		Replicates per Group		many replicates there were and how many organisms were in each exposure group.			
		Conti	nued on next pa	nge			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation: Duration:	Streufert, J. the Missouri Overall Dura	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges (Chironomus plumosus). Transactions of the Missouri Academy of Science 14:33-40. Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus plumosus; Larvae					
Health Outcome:	Developmen	t/Growth					
HERO ID.	813673	(DEHP)					
Domain	013073	Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. Organisms were fed 0.12g of dog candy daily until pupation. It was not reported how many organisms were in each test chamber.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-adult emergence-development/growth.			
	Metric 18:	Consistency of Outcome Assessment	High	Pupal skin cast offs were counted and removed daily until there were 2 consecutive days without finding a pupal cast off since the start of emergence.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Analysis of variance, arcsine transformation, and the least significance test were used to analyze data.			
	Metric 22:	Reporting of Data	High	Control and exposure response data were reported in Table 3 and were adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	This portion	of the evaluation was on the chronic toxicity	of DEHP on mi	dge emergence. Development and growth was selected as the outcome of interest.			

Overall Quality Determination

Medium

Study Citation: Duration:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus plumosus; Larvae					
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1332972					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.		
	Metric 2:	Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.		
	Metric 3:	Test Substance Purity	Low	No purity was reported.		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4.	Negative Controls	High	Solvent controls (ethanol) were used at 0.12 ml/L		
	Metric 5:	Negative Control Response	High	Control responses are reported for emergence from chronic exposures.		
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	High	The chronic exposures were conducted with a flow-through system for continual renewal		
		Preparation	TT 1	of the chemical. The setup and flow-rate were described well on page 32/62.		
	Metric 8:	Consistency of Exposure	High	The exposure administration appeared consistent among treatments and the control.		
	Metric 9:	Measurement of Test Substance	Medium	GC was used to verify the concentrations from the chronic exposure on page 25/62.		
	Metric 10:	Exposure Duration and Frequency	High	The chronic exposures for midge emergence ranged from 20-40 days for hydrosoil sub- strate and 20 - 35 days for sand substrate.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The measured concentrations are reported in tables within the results section beginning on page 35/62.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The chronic exposures are mostly below the published solubility value published in the final scope for DEHP (0.27 mg/l).		
Domain 4. Test Organia						
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life		
	Weute 15.	Test organism characteristics	Ingh	stages were identified.		
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the chronic exposures.		
	Metric 15.	Conditions Number of Organisms and	Low	Authors began each treatment and control group with 100 1st instar larvae for the		
	Metric 15.	Replicates per Group	Low	chronic emergence bioassay. The replication and housing groups for this work were not well described.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the chronic exposures.		
		Conti	nued on next pa	ge		
		1	Page 374 of 95 9	3		

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1332972 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology Medium No significant differences in emergence were observed for the chronic exposures. Consistency of Outcome Metric 18: High The outcomes appear to be reported consistently. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: High Nothing was reported to indicate differences among treatment groups with environmen-Design and Procedures tal conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information to suggest differences among groups related to health outcomes. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High The percent data was arc sin square-root transformed and Least Significant Difference tests were performed. Metric 22: Reporting of Data Medium Emergence is reported in total numbers for each concentration, compound, and day of exposure in tables presented in the results section. Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported. Additional Comments: None

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus plumosus; Adult					
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1332972					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.		
	Metric 2:	Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.		
	Metric 3:	Test Substance Purity	Low	No purity was reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Solvent controls (ethanol) were used at 0.12 ml/L.		
	Metric 5:	Negative Control Response	High	Control responses are reported (table 10 and 11) for the reproductive study.		
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The reproductive study was conducted with a flow-through system for continual renewal of the chemical. The setup and flow-rate was described well on page 32/62.		
	Metric 8:	Consistency of Exposure	High	The exposure administration appeared consistent among treatments and the control.		
	Metric 9:	Administration Measurement of Test Substance	Medium	GC was used to verify the concentrations from the reproduction exposure on page 25/62.		
	Metric 10:	Exposure Duration and Frequency	High	The reproduction study was conducted with previously exposed adults from the previously chronic study.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The measured concentrations are reported in tables within the results section beginning on page 35/62.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The exposures for the reproduction study were all under solubility limit except for one concentration at 0.362 mg/l with the published solubility value in the final scope for DEHP (0.27 mg/l).		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.		
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the chronic exposures.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The replication and housing groups for this work were not well described.		
		Replicates per Gloup				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the chronic exposures.		
		Cont	inued on nex	t page		

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Adult **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1332972 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology Medium No significant differences in emergence were observed for the chronic exposures.From Page 34/62 - "Studies using both sand and hydrosoil substrates showed DEHP had no effect on midge reproduction.Likewise, neither reproductive parameter was affected by DEHP concentrations up to 362 ug/l when sand was used as a substrate (Table 11)." Metric 18: Consistency of Outcome High The outcomes appear to be reported consistently. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High Nothing was reported to indicate differences among treatment groups with environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information to suggest differences among groups related to health outcomes. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High The percent data was arc sin square-root transformed and Least Significant Difference tests were performed. Metric 22: Reporting of Data High The reproductive parameters are presented in Table 10 and 11. Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination

High

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity Di-ethylhexyl phthalate (DEHP) 3859131				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	Metric 1:	Test Substance Identity	High	The chemical was identified by name (di(2-ethylhexyl) phthalate, DEHP) and CASRN (117-81-7).	
	Metric 2:	Test Substance Source	Low	The source was identified (Sigma-Aldrich, USA), but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design			TT 1		
	Metric 4: Metric 5:	Negative Control Response	High	A concurrent solvent control (0.01% ethanol) was used. The biological response of the solvent control group was reported and adequate (repre- sented in bar graphs by letter C of Figures 1 through 5).	
	Metric 6:	Randomized Allocation	Medium	Authors reported that groups of 20 larvae were selected randomly and exposed to aqueous solutions of DEHP without sediment.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the nominal concentrations were not verified during the study.	
	Metric 8:	Consistency of Exposure	Low	Details of the exposure administration were not reported.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Metric 10:	Exposure Duration and Frequency	Low	Exposure durations for the assessment of the molecular effects were hardly described in the methods. The results (Figures 1 through 5) made it more clear to understand the exposure duration because the figures show that data were collected 24, 48, 72, and 96 h post-exposure and for a group at 24 h post-exposure followed by 24 h of depuration. OECD Test No. 235: Chironomus sp., Acute Immobilization Test describes a range of concentrations of the test substance in water-only vessels for a period of 48 h. Given that the authors assessed molecular effects and not mobilization or mortality, the exposure duration of 24h is also appropriate.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable for the molecular effects. While the actual number of exposure groups was not reported in the methods, except for GST activity, the authors only reported that gene expression analyses were carried out at four doses: 10^{-3} , 10^{-2} , 10^{-1} , and $1 \mu g/L$. GST activity was carried out at two doses: 1 and $10^{-3} \mu g/L$.	
		Con	tinued on next pa	ge	

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Environmental Hazard Evaluation

HERO ID: 3859131 Table: 1 of 2

rrero, Ó., evant conc erall Dura uatic (fres vertebrate; echanistic- ethylhexy 59131	Morcillo, G., Planelló, R. (2017). Transcri entrations of di(2-ethylhexyl) phthalate (DI tion: 0 - 4 days (0-96h); Exposure Duratior hwater); Water; Not determined by study an Arthropods; <i>Chironomus riparius</i> ; Larvae Biomarkers (exposure and effect)-Receptor l phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	ptional deregula EHP). PLoS ON 1: 0 - 4 days (0-9 uthors (i.e., chen binding/ regulat Rating Medium	tion of genetic biomarkers in Chironomus riparius larvae exposed to ecologically E 12(2):e0171719. 6h) nical of interest in exposure water, but unable to determine exact uptake route) tion of receptor activity Comments
erall Dura uatic (fres vertebrate; echanistic- ethylhexy 59131	tion: 0 - 4 days (0-96h); Exposure Duration hwater); Water; Not determined by study an Arthropods; <i>Chironomus riparius</i> ; Larvae Biomarkers (exposure and effect)-Receptor l phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	n: 0 - 4 days (0-9 uthors (i.e., chen binding/ regular Rating Medium	(h) (h) (c) of interest in exposure water, but unable to determine exact uptake route) (c) of receptor activity (C) Comments
uatic (fres vertebrate; echanistic- ethylhexy 59131 etric 12:	hwater); Water; Not determined by study at Arthropods; <i>Chironomus riparius</i> ; Larvae Biomarkers (exposure and effect)-Receptor I phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	uthors (i.e., chen binding/ regular Rating Medium	nical of interest in exposure water, but unable to determine exact uptake route) tion of receptor activity Comments
vertebrate; echanistic- ethylhexy 59131 etric 12:	Arthropods; <i>Chironomus riparius</i> ; Larvae Biomarkers (exposure and effect)-Receptor l phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	binding/ regulat Rating Medium	tion of receptor activity Comments
ertebrate; echanistic- ethylhexy 59131 etric 12:	Arthropods; <i>Chironomus riparius</i> ; Larvae Biomarkers (exposure and effect)-Receptor l phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	binding/ regulat Rating Medium	tion of receptor activity Comments
echanistic- ethylhexy 59131 etric 12:	Biomarkers (exposure and effect)-Receptor l phthalate (DEHP) <u>Metric</u> Testing at or Below Solubility Limit	binding/ regulat Rating Medium	tion of receptor activity Comments
ethylhexy 59131 etric 12:	l phthalate (DEHP) Metric Testing at or Below Solubility Limit	Rating	Comments
59131 etric 12:	Metric Testing at or Below Solubility Limit	Rating	Comments
etric 12:	Metric Testing at or Below Solubility Limit	Rating	Comments
etric 12:	Testing at or Below Solubility Limit	Medium	
		meanum	All concentrations, except for $10^{3} \mu g/L$ in the GST activity assay, were below the DEHP water solubility limit (270 ug/L). The authors reported using ethanol as the solvent at 0.01%, but the authors did not offer details on whether 0.01% ethanol was sufficient to dissolve 1,000 ug/L DEHP.
etric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported. Moreover, the authors reported using the fourth instar larvae. However, OECD Test No. 235: Chironomus Acute Immobilization Test indicates that the first instar larvae of Chironomus riparius are most suitable for the acute waterborne toxicity studies with Chironomus because they have been shown to be the most sensitive larval stage. Further, the fist instar is free swimming and therefore not stressed by the absence of sediment.
etric 14:	Acclimatization and Pretreatment Conditions	High	Study authors reported how cultures were maintained. There was no reason to believe that the pretreatment conditions were not the same across treatment groups.
etric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to charac- terize molecular effects: The authors reported using groups of 20 larvae for DEHP solutions in four independent experiments, and groups of five surviving larvae were randomly selected and used for RNA or protein extraction.
aant			
etric 16:	Adequacy of Test Conditions	Low	Environmental conditions before the exposure were described, but during exposure, they were not sufficiently reported to evaluate if they were adequate.
etric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (semi-quantitativeRT-PCR and GST activity) wa clearly reported for the outcome of interest (molecular effects).
etric 18:	Consistency of Outcome Assessment	High	There is no evidence to suggest that the molecular effects were not assessed consistently across study groups.
riable Cor	otrol		
etric 19.	Confounding Variables in Test	High	The study did not provide enough information to allow a comparison of environmental
	Design and Procedures		conditions.
etric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
and An-1-			
and Analy etric 21:	Statistical Methods	High	Statistical analysis was performed and described adequately (ANOVA, followed by Games Howell's or Bonferroni's post hoc tests for normally distributed data or Knuckal-
neetiettiineettiineettiineettiineettiineettiineettiineettiineettiineettiineettiineettiineettiineettiineettiinee	ric 14: ric 15: ent ric 16: ric 17: ric 18: able Cor ric 19: ric 20: und Analy ric 21:	ric 14: Acclimatization and Pretreatment Conditions ric 15: Number of Organisms and Replicates per Group ent ric 16: Adequacy of Test Conditions ric 17: Outcome Assessment Methodology ric 18: Consistency of Outcome Assessment able Control ric 19: Confounding Variables in Test Design and Procedures ric 20: Outcomes Unrelated to Exposure und Analysis ric 21: Statistical Methods	ric 14: Acclimatization and Pretreatment Conditions ric 15: Number of Organisms and Replicates per Group Medium Replicates per Group Tic 16: Adequacy of Test Conditions ric 17: Outcome Assessment Methodology ric 17: Outcome Assessment Methodology High ric 18: Consistency of Outcome Assessment High Design and Procedures ric 20: Outcomes Unrelated to Exposure Medium

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Environmental Hazard Evaluation

HERO ID: 3859131 Table: 1 of 2

		continu	ued from previ	ous page	
Study Citation:	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa, Species, Age:	Invertebrate: Arthropode: Chiropomus ringrius: Larvae				
Health Outcome	Mechanistic Biomarkers (exposure and effect) Recentor hinding/ regulation of recentor activity				
Chemical:	Di. ethylheyyl phthalate (DEHP)				
HERO ID:	3859131	() philadae (DDHI)			
Domain		Metric	Rating	Comments	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Figures 1 through 5).	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	The goal of the study was to analyze the molecular effects of DEHP exposure on Chironomous riparius larvae by evaluating the enzyme activity of glutathione S-transferase (GST) and transcriptional activity of genes related to crucial cell systems. The authors also conducted survival tests to examine mortality. This form was used to evaluate the molecular effects including the enzyme activity of glutathione S-transferase (GST) and transcriptional activity of genes.				

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae Mortality Di-ethylhexyl phthalate (DEHP)					
Domain	5057151	Metric	Rating	Comments		
Domain 1: Test Substand	ce		8			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name (di(2-ethylhexyl) phthalate, DEHP) and CASRN (117-81-7).		
	Metric 2:	Test Substance Source	Low	The source was identified (Sigma-Aldrich, USA), but the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not reported for the larval survival studies para- graph in the methods (only concentration range of 10^{-3} to $10^{5} \mu g/L$ was reported) or		
	Metric 5:	Negative Control Response	Uninformative	In the results, which were only described in the text (no tables or figures). A concurrent negative control group was not reported in the methods (only concentration range of 10^{-3} to $10^{5} \mu g/L$ was reported) or in the results, which were only described in the text (no tables or figures).		
	Metric 6:	Randomized Allocation	Medium	Authors reported that groups of 20 larvae were selected randomly and exposed to an aqueous solutions of DEHP without sediment.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the nominal concentrations were not verified during the study.		
	Metric 8:	Consistency of Exposure Administration	Low	Details of the exposure administration were not reported. Moreover, DEHP degrades overtime, and authors did not mention replacing DEHP during the exposure duration of up to 96 h.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	Uninformative	There was ambiguity in exposure durations for each of the tests, and most importantly, some exposures were only for 24 h. The authors conducted a waterborne exposure without sediment for 24h and for "longer treatments (48 to 96 h)" at the four lowest doses $(10^{-3}$ to 1 μ g/L) as per methods and description in the results. No other details were reported. This study was more similar to an acute waterborne toxicity test than a sediment toxicity test. Rationale for ranking: According to EPA TG OCSPP 850.1790 Chironomid Sediment Toxicity Test, Part I (the aqueous exposure test) the exposure period is 14 days. OECD Test No. 235: Chironomus sp., Acute Immobilization Test describes a range of concentrations of the test substance in water-only vessels for a period of 48 h.		
		(Continued on next page	·· ·		

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Diethylhexyl Phthalate

Study Citation:	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically								
Duration	relevant con	centrations of di(2-ethylhexyl) phthalate (DEH ation: $0 - 4$ days (0-96h): Exposure Duration: (P). PLoS ONE 12(2): - 4 days (0-96h)	e0171719.					
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae							
Media, Path:	1								
Taxa, Species, Age:	Invertebrate								
Health Outcome:	Mortality								
Chemical:	Di-ethylhexy	yl phthalate (DEHP)							
	3839131			2					
Domain	N. 4 . 11	Metric	Rating	Comments					
	Metric 11:	Spacing of Exposure Levels	Medium	For the larval survival study, the actual number of exposure groups was not reported. The authors only reported the range as 10^{-3} to $10^{5} \mu g/L$, and they indicated that the four lowest doses were 10^{-3} to $1 \mu g/L$. So, one can deduce that the exposure groups for the survival study were 10^{-3} , 10^{-2} , 10^{-1} , 1 , 10^{-2} , 10^{-3} , 10^{4} , and $10^{5} \mu g/L$.					
	Metric 12:	Testing at or Below Solubility Limit	Low	Several concentrations (nominal concentrations ranged from 10^{-3} to $10^{5} \mu g/L$) exceeded the DEHP water solubility limit (270 ug/L). The authors reported using ethanol as the solvent at 0.01%, but the authors did not offer details on whether 0.01% ethanol was sufficient to dissolve 100,000 ug/L DEHP.					
Domain 4. Test Organ	ism								
Domani ii Test Organ	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported. Moreover, the authors reported using fourth instar larvae. However, OECD Test No. 235: Chironomus Acute Immobilization Test indicates that the first instar larvae of Chironomus riparius are most suitable for the acute waterborne toxicity studies with Chironomus because they have been shown to be the most sensitive larval stage. Further, the first instar is free swimming and therefore not stressed by the absence of sediment.					
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Study authors reported how cultures were maintained. There is no reason to believe that the pretreatment conditions were not the same across treatment groups.					
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The authors reported using groups of 20 larvae for DEHP solutions in four independent experiments. OECD Test No. 235: Chironomus Acute Immobilization Test indicates that at least 20 larvae, preferably divided into four groups of five larvae each, should be used for each test concentration and for controls. Therefore, the number of organisms per treatment group was exceeded, and the replicates were as expected.					
Domain 5: Outcome A	ssessment								
Domain 51 Guteoine 1	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions before the exposure were described, but during exposure, they were not sufficiently reported to evaluate if they were adequate.					
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (calculation of survival rates after 24, 48, 72, or 96 h.) was partially reported for the outcome of interest (survival/mortality rate).					
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.					
Domain 6: Confoundi	ng / Variable Co	ntrol							
20man o. Comoditul	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.					

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Diethylhexyl Phthalate

		CO	ntinued from previous	page	
Study Citation:	Herrero, Ó.,	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in Chironomus riparius larvae exposed to ecologically			
Duration: Exposure Route, Media, Path:	relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus riparius; Larvae			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	3859131				
Domain	Metric		Rating	Comments	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis for survival data were not described in detail. Tables and figures were not reported. The results in the text indicate that no significant mortality in survival data was observed and that survival rates were very close to 100%. It is unclear if the "significant" was referring to statistical significance or a descriptive term.	
	Metric 22:	Reporting of Data	Uninformative	Data representation was insufficient and inadequate.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability occurred.	
Additional Comments:	The goal of the study was to analyze the molecular effects of DEHP exposure on Chironomous riparius larvae by evaluating the enzyme activity of glutathione S-transferase (GST) and transcriptional activity of genes related to crucial cell systems. The authors also conducted survival tests to examine mortality. This form was used to evaluate the mortality data which lacked tabulated results or a figure in the results. The authors only described the results in the text, and in the results, there is additional or inconsistent information regarding the actual exposure durations tested compared to the information of				

the survival tests in the methods section.

Overall Quality Determination

Uninformative

Study Citation:	Park, K., Kv	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during Chironomus riparius				
Duration	development	development. Science of the Total Environment 470-471:1003-1011. Overall Duration: 0 - 4 days (0-96b): Exposure Duration: 0 - 4 days (0-96b)				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)	including redux bi	ology)		
HERO ID:	2519014					
Domain	Metric Rating Comments					
Domain 1: Test Substan	ce		т			
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by name only.		
	Metric 3:	Test Substance Durity	Low	The purity/grade of the DEHP was not reported.		
	Wiettie 5.	Test Substance Fullty	Low	The purity grade of the DETH was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	There were two separate studies that were reported on in this reference. The first portion looked at the effect of temperature alone, and the second portion looked at the effect of DEHP and temperature. The control for the second portion of the study was reported to be 0.5mg/L DEHP exposure at 20C. This is not a true negative control. However, the first portion of the study had an experimental group conducted at 20C only, so it is possible that a comparison could be made. It did not appear that the two studies were run concurrently, and direct comparisons between the studies were not made in the		
	Metric 5:	Negative Control Response	Medium	It is possible that negative control comparisons could be made using the first portion of the study that looked at the effect of temperature alone since the second portion of the study considered a DEHP exposure of 0.5mg/L at 20C was considered the negative control. It is important to note that this is not a true negative control since there is still exposure to DEHP. If the first portion of the study is to be used for negative control results, data can be found in Fig. 2, 3, and 4.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the C. riparius larvae were allocated into study groups.		
Domain 3. Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Low	It was not reported how the DEHP concentration was prepared. Tests were conducted in		
		Preparation	2011	300mL crystallizing dishes and treated with 0.5mg/L DEHP. Little other details regard- ing the test system were provided.		
	Metric 8:	Consistency of Exposure Administration	Low	All exposures were conducted in 300mL crystallizing dishes, and a 16L:8D photoperiod was used. The exposure duration was for 24h. Little other information was provided, so it was difficult to determine how consistent the exposure administration was.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentration was measured at any point in the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 24h. This appeared adequate to observe a response.		
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level, as the study goal was not to observe a dose response,		
		Spacing of Exposure Levels		but to compare exposures at different temperatures.		
	Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 2519014 Table: 1 of 1

		conti	nued from previ	ious page		
Study Citation: Duration: Exposure Route, Media, Path:	Park, K., Ky development Overall Dura Aquatic (free	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during Chironomus riparius development. Science of the Total Environment 470-471:1003-1011. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Health Outcome:	Mechanistic	-Cell signaling/function-Oxidative stress (in	ncluding redox bi	iology)		
Chemical: HERO ID:	Di-ethylhexy 2519014	yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
	Metric 12:	Testing at or Below Solubility Limit	Low	The test concentration appeared to be above the water solubility limit for DEHP. The testing at different temperatures may have also affected the water solubility. It is unclear if a vehicle solvent was used.		
Domain 4: Test Organi	sm					
	Metric 13:	Test Organism Characteristics	High	The organisms were provided from adults reared in the performing laboratory. The original strain was provided by the Korea Institute of Toxicology. 4th instar individuals approximately 11-13 days old were used for this portion of the study.		
	Metric 14:	Acclimatization and Pretreatment	High	Test organisms were cultured under similar conditions to the testing conditions.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	It was reported that 13 fourth instar larvae were used in this portion of the study and that there were 3 replicates, but it is unclear how many organisms were in each replicate.		
Domain 5: Outcome A	ssessment					
Domain 5. Outcome A	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were reported to be raised in M4 medium in 500mL crystallizing dishes. They were fed fish food at a rate of 0.5mg per larva per day. 1cm of sand was placed at the bottom of the dish. Larvae were reared at 20C with a relative humidity of 70% with a photoperiod of 16L:8D. Testing temperatures were 10, 20, and 30C. Continuous aeration was provided. Water quality parameters were not reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mechanistic outcomes in terms of mRNA expressions of endocrine signaling genes and antioxidant genes.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. All organisms were assessed 24h after exposure for endocrine related genes and oxidative stress related genes.		
Domain 6: Confoundin	og / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presen	ntation and Anal	lysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported in section 2.5 "Data analysis."		
		Conti	inued on next pa	nge		
				0		

Diethylhexyl Phthalate

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 2519014 Table: 1 of 1

		continued from previo	bus page			
Study Citation:	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during Chironomus riparius development. Science of the Total Environment 470-471:1003-1011.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Health Outcome:	Mechanistic-Cell signaling/function-Oxida	ative stress (including redox bi	ology)			
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	2519014					
Domain	Metric	Rating	Comments			
	Metric 22: Reporting of Data	Medium	Data was provided for the exposures in Fig. 5. Negative control data for 20C without DEHP exposure could be obtained from the temperature only portion of the study. This is a separate test from the DEHP exposure portion of the study. This data is presented in Fig. 2, 3, and 4.			
	Metric 23: Explanation of Unexpected	Outcomes Medium	is a limitation when it comes to unexpected outcome. Study authors did provide mea- sures of variability within each test.			
Additional Comments:	This evaluation was on the effect of DEHP as well as varying temperature on C. riparius 4th instar larvae oxidative stress and and endocrine gene expression. There were two portions to this study. One portion involved an assessment of varying temperatures on C. riparius. The other portion involved an assessment of the effect of DEHP and varying temperature on C. riparius. These appeared to be two different studies that may not have been run concurrently. The DEHP portion of the study did not have a true concurrent negative control. For this portion of the study, study authors reported the negative control was 0.5mg/L DEHP at 20C. However, the temperature only portion of the study did have test group at 20C without DEHP. It is possible that this could be used as a comparator in the study with DEHP. The two different studies were not compared directly in the paper by study authors.					
Overall Qualit	y Determination	Medium				

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Study Citation:	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in Chironomus riparius exposed to di-2-ethylhexyl phthalate (DEHP). Journal of					
Duration:	Environmen Overall Dura	tal Biology 25(3):259-261. ation: > 21 days: Exposure Duration: > 21 d	lavs			
Exposure Route,	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Embryo					
Health Outcome:	Developmen	t/Growth				
Unemical: HFRO ID:	681990	yi phinaiale (DEHP)				
Domain	001770	Metric	Rating	Comments		
Domain 1: Test Substand	ce	incure	Rung	Comments		
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2. Test Design						
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
		Administration	6	I		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and the spacing of exposure levels. This may have contributed to the poor dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 1: Test Organis	m					
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
		Conditions				
	Metric 15:	Number of Organisms and	Low	It was unclear if the number of replicates was equal between treatments or if the number		
		Replicates per Group		of organisms was similar.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.		
		Contir	nued on next pa	ge		

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Diethylhexyl Phthalate

	continued from previous page						
Study Citation:	Kim, E. J.,	Lee, S. K. (2004). Reduced viability of I	F1 egg ropes in	Chironomus riparius exposed to di-2-ethylhexyl phthalate (DEHP). Journal of			
	Environmen	Environmental Biology 25(3):259-261.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	ly authors (i.e., cl	hemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus riparius; Embryo					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	681990						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.			
Domain 6: Confounding	g / Variable Co	ntrol					
_	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	e				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties were identified in how the study characterized unexpected out- comes.			
Additional Comments:	The authors	characterized all outcomes as reproductive e	effects. This forn	n accounts for the emergence endpoint.			

Overall Quality Determination

Medium

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HERO ID: 681990 Table: 2 of 2

Study Citation:	Kim, E. J.,	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in Chironomus riparius exposed to di-2-ethylhexyl phthalate (DEHP). Journal of					
Duration	Environmental Biology $25(3):259-261$.						
Exposure Route	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media. Path:							
Taxa, Species, Age:	Invertebrate	: Arthropods: Chironomus riparius: Embryo					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhex	Di-ethylbexyl phthalate (DEHP)					
HERO ID:	681990	()					
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	ice						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Cl	paracterization						
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately proper			
	Methe 7.	Preparation	Low	test concentrations.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
		Administration	8	I			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	There were minor limitations regarding the number of exposure groups and the spacing			
		Spacing of Exposure Levels		of exposure levels. This may have contributed to a poor dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	sm						
U	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	It was unclear if the number of replicates was equal between treatments or if the number of organisms was similar			
		Replicates per Gloup					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest.			
		Contin	nued on next pa	nge			

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Kim, E. J., Environment	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in Chironomus riparius exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.				
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stud	y authors (i.e., cł	nemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus riparius; Embryo				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	681990					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not		
		Assessment		reported.		
Domain 6: Confounding	g / Variable Coi		TT: 1			
	Metric 19:	Confounding variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes.		
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties were identified in how the study characterized unexpected outcomes.		
Additional Comments:	The authors characterized all outcomes as reproductive effects.					

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology						
Duration: Exposure Route, Media. Path:	74(6):1179-1185. Overall Duration: Not-reported; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	681634						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).			
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Cha	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail			
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured; only nominal concentrations were re-			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, $) was used to aid in dissolution of the test compound.$			
Domain 4: Test Organisr	n						
-	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects (20 larvae per test vessel, 0 replicates per test concentration)			
		Replicates per Group		ize toxicological effects (20 faivae per test vessel, 9 replicates per test concentration).			
Continued on next page							

HERO ID: 681634 Table: 1 of 3

		conti	nued from p	previous page		
Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1170-1185					
Duration:	Overall Dura	Overall Duration: Not-reported: Exposure Duration: Not-reported				
Exposure Route,	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Health Outcome:	Mortality	• · · · ·				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	681634					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures	6			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were reasonably described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.		
Additional Comments:	This study u	tilized a developmental based study duration	on.			
Overall Quali	ty Detern	nination	High			

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HERO ID: 681634 Table: 2 of 3

Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.						
Duration: Exposure Route, Media. Path:	Overall Duration: Not-reported; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae						
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	681634						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	e						
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).			
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	reatorization						
Domain 5. Exposure Cha	Metric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-			
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured; only nominal concentrations were re-			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, $) was used to aid in dissolution of the test compound.$			
Domain 4: Test Organisr	<u></u>						
Domani 4. Test Organisi	Metric 13.	Test Organism Characteristics	Low	The source of the test animals was not reported			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects (20 larvae per test vessel, 9 replicates per test concentration).			

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 681634 Table: 2 of 3

		conti	nued from p	previous page		
Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.					
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Health Outcome:	Developmen	nt/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	681634					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
Domain 6: Confoundin	g / Variable Co	ntrol Confounding Variables in Test	High			
	Metric 19:	Design and Procedures	підп	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Presen	tation and Anal	lysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reasonably described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.		
Additional Comments:	This study u	tilized a developmental based study duratio	on. Growth a	nd development time were evaluated in this form.		
Overall Quali	iy Deterr	mnation	пign			

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HERO ID: 681634 Table: 3 of 3

Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185						
Duration: Exposure Route, Media. Path:	Overall Duration: Not-reported; Exposure Duration: Not-reported Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate:	Arthropods: Chironomus riparius: Larvae					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	681634						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).			
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.			
Demain 2. Test Design							
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured; only nominal concentrations were re- ported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, $) was used to aid in dissolution of the test compound.$			
Domain 4: Test Organis	m						
_ shan ii rost organisi	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
		Conditions		· · · ·			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects (20 larvae per test vessel, 9 replicates per test concentration).			

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 681634 Table: 3 of 3

		conti	nued from p	previous page		
Study Citation:	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in Chironomus riparius. Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.					
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus riparius; Larvae					
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	681634					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment	-			
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Presen	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were reasonably described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.		
Additional Comments:	This study u	tilized a developmental based study duration	on. This form	addresses the sex ratio outcome.		
Overall Quali	ty Detern	nination	High			

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. M., L larvae expose Overall Dura Aquatic (fres Invertebrate; Developmen Di-ethylhexy 492760	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere 65(6):1074-1081. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae Development/Growth Di-ethylhexyl phthalate (DEHP) 492760					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Medium	The test chemical was identified by name.			
	Metric 2:	Test Substance Source	High	The source was stated as Fluka.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design	Matric 4:	Nagativa Controls	Low	It was upplaar if outbors used a solvent control, although, use of a control was reported			
	Meure 4.	Negative Controls	LOw	and shown in Figure 5.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate and shown in Figure 5.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set-up was explained; the addition of the chemical to the beakers had few details.			
	Metric 8:	Consistency of Exposure Administration	Low	Few details of the exposure administration were reported. There was no mention of how the controls were prepared.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	This was a 48 hour exposure for assessing body weight.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations and a control were utilized in this study. The DEHP concentrations cover two orders of magnitude. Additional doses could have been added to the low end for a better dose response representation.			
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentrations stated in Figure 5 and in the text for body weight are above the solubility limits for DEHP in water (5 mg/L, 50 mg/L).			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The source of the test organisms was stated.			
	Metric 14:	Acclimatization and Pretreatment	Low	The test organisms were not acclimatized to the test beakers prior to chemical exposure.			
	Matria 15.	Conditions	Low	There were 10 test organisms per backer. The sumber of realisate backers ('f)			
		Replicates per Group	LOW	chemical treatment was not stated.			

Domain 5: Outcome Assessment

Environmental Hazard Evaluation

HERO ID: 492760 Table: 1 of 2

bosed to various environmental pollutants: A puration: 0 - 4 days (0-96h); Exposure Durati freshwater); Water; Not determined by study ate; Arthropods; <i>Chironomus tentans</i> ; Larvae nent/Growth exyl phthalate (DEHP) <u>Metric</u>	potential biomark ion: 0 - 4 days (0-9 authors (i.e., chen e	ter of freshwater monitoring. Chemosphere 65(6):1074-1081. 96h) nical of interest in exposure water, but unable to determine exact uptake route)
Puration: 0 - 4 days (0-96h); Exposure Durati freshwater); Water; Not determined by study ate; Arthropods; <i>Chironomus tentans</i> ; Larvac nent/Growth exyl phthalate (DEHP) <u>Metric</u>	ion: 0 - 4 days (0-9 r authors (i.e., chen	96h) nical of interest in exposure water, but unable to determine exact uptake route)
freshwater); Water; Not determined by study ate; Arthropods; <i>Chironomus tentans</i> ; Larvae nent/Growth exyl phthalate (DEHP) <u>Metric</u>	Pating	nical of interest in exposure water, but unable to determine exact uptake route)
tte; Arthropods; <i>Chironomus tentans</i> ; Larvae nent/Growth exyl phthalate (DEHP) <u>Metric</u>	Paties	
tte; Arthropods; <i>Chironomus tentans</i> ; Larvae nent/Growth exyl phthalate (DEHP) <u>Metric</u>	Pating	
nent/Growth exyl phthalate (DEHP) Metric	Dating	
Metric	Dating	
Metric	Dating	
Metric	Dating	
A degree of Test Condition-	Kating	Comments
Adequacy of fest Conditions	Medium	The DO and pH of the test water were not stated. Other conditions were explained satis factorily.
: Outcome Assessment Methodology	High	The outcome assessment methodology reported how fresh weights and dry weights wer obtained.
: Consistency of Outcome Assessment	High	The outcome methodology was conducted at 48 hr after the start of the exposure.
Control		
: Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
Design and Procedures		
: Outcomes Unrelated to Exposure	Medium	There was no information to suggest difference in animal attrition among treatments.
nalvsis		
: Statistical Methods	High	The authors utilized the parametric t test to determine significant differences.
: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in Figure 5.
: Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unex- pected outcomes. A trend was reported but had high variability.
$\frac{7}{10}$ $\frac{1}{10}$	 8: Consistency of Outcome Assessment 8: Consistency of Outcome Assessment 9: Confounding Variables in Test Design and Procedures 0: Outcomes Unrelated to Exposure Analysis 1: Statistical Methods 2: Reporting of Data 3: Explanation of Unexpected Outcomes may be warranted for the units of DEHP in thi tt endpoints (with supposedly the same doses 	 6. Outcome Assessment Methodology High 8: Consistency of Outcome High Assessment Control 9: Confounding Variables in Test High Design and Procedures 0: Outcomes Unrelated to Exposure Medium Analysis 1: Statistical Methods High 2: Reporting of Data High 3: Explanation of Unexpected Outcomes Medium

... continued from previous page

Study Citation: Duration: Exposure Route, Media Path:	Lee, S. M., I larvae expos Overall Dura Aquatic (fre	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere 65(6):1074-1081. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate Mechanistic Di-ethylhex	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP)					
HERO ID:	492760						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce Matria 1:	Toot Substance Identity	Madium	The test shaminal was identified by name			
	Metric 1: Matria 2:	Test Substance Identity	Lich	The test substance was obtained from Fluke			
	Metric 2.	Test Substance Source	Low	The numity on d/on one do of the test substance were not reported.			
	Metric 5:	Test Substance Purity	LOW	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
2 onian 21 1000 2 oorgi	Metric 4:	Negative Controls	Low	Use of a solvent control was not explicitly stated; a 0 mg/L treatment was included in Figure 2.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set-up was explained; the addition of the chemical to the beakers had few reported details.			
	Metric 8:	Consistency of Exposure Administration	Low	Few details of the exposure administration were reported. There was no mention of how controls were prepared.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	Medium	There was a 24 hr exposure period for the gene expression endpoint.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations and a control were utilized in this study. The DEHP concentrations cover two orders of magnitude. Additional doses could have been added to the low end for a better dose response representation			
	Metric 12:	Testing at or Below Solubility Limit	Low	It was unclear whether doses exceeded solubility in the gene expression analysis as the units in Figure 2 state both mg/L (which exceed approximate solubility of 0.3 mg/L) and ug/L for DEHP were used. The text states ug/L for gene expression results.			
Domain 4 [.] Test Organis	m						
2 children in root organio	Metric 13:	Test Organism Characteristics	High	The source of C. tentans was stated.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 10 test organisms per beaker. The number of replicate beakers (if any) per chemical treatment was not stated. It is unclear if the experiment was performed thrice.			
Domain 5: Outcome As	sessment						
2 sinain 5. Outcome Ab	Metric 16	Adequacy of Test Conditions	Medium	The DO and pH of water were not stated. Other conditions were explained satisfactorily			
	Metric 17:	Outcome Assessment Methodology	High	The outcome methodology for gene expression analysis was well described.			
Continued on next page							

HERO ID: 492760 Table: 2 of 2

		contin	ued from previ	ous page			
Study Citation:	Lee, S. M., I larvae expos	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere 65(6):1074-1081.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Mechanistic	-Cell signaling/function					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	492760						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment methodology was carried out at 24 hr for all exposure groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences in animal attrition among the different groups.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	The authors utilized parametric t test to determine significant differences.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group in Figure 2. There was inconsistent use of units for DEHP in Figure 2 and in the text $(0.5, 5, 50 \text{ ug/L vs } 0.5, 5, 50 \text{ mg/L})$.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unex- pected outcomes (e.g. high variation in group receiving the highest dose of DEHP).			
Additional Comments:	The doses u inconsistenc for both end	The doses utilized for DEHP are inconsistent in units (unclear which units are correct). Caution may be warranted for the units of DEHP in this study as inconsistency in figures and text between doses (units) are stated for mechanistic endpoints vs weight endpoints (with supposedly the same doses utilized for both endpoints, i.e. the 1/10, 1/100, and 1/1000th of the 24 hr LC50).					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Monsanto, (Overall Dura Aquatic (fre	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to Chironomus tentans. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae Immobilization					
Taxa, Species, Age:	Invertebrate						
Health Outcome:	Immobilizat						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1335360						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only. A CASRN number is present on the cover sheet and written on a page, but it does not appear to be original to when the document was formed.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.			
	Metric 5:	Negative Control Response	High	All solvent control animals were immobilized at 48hr (page 7/10). The treatment con- centration (10 mg/L DEHP) and control were both 0% immobilized.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure	Low	Not enough information was reported to adequately assess this metric.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only one concentration was used with no effects reported.			
	Metric 12:	Testing at or Below Solubility Limit	Low	The test concentration was 10mg/L, which is over the solubility reported in the final Scope for DEHP (0.2 mg/L).			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.			
		Tepheneo per Group		-			

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

		contin	ued from previou	us page		
Study Citation:	Monsanto, (1	983). Acute toxicity of di (2-ethylhexyl) ph	nthalate to Chirono	omus tentans.		
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-96	h)		
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Immobilizati	on				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1335360					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not		
		Assessment		reported.		
Demain (; Canfanalia	Werdelle Com	4 m 1				
Domain 6: Confounding	/ variable Con	Confounding Variables in Test	High			
	Metric 19:	Design and Presedures	пign	There were no reported differences among the study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Presenta	ation and Analy	vsis				
	Metric 21:	Statistical Methods	N/A	Only one concentration was assessed.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	y Detern	nination	Medium			

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Study Citation: Duration: Exposure Route, Media, Path:	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to Chironomus tentans. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus tentans; Larvae			
Health Outcome:	Di-ethylbey	al phthalate (DEHP)			
HERO ID:	1335360				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ice				
	Metric 1:	Test Substance Identity	Low	Chemical identified by name only. CASN number is present on cover sheet and written on page, but do not appear to be original to when document was formed.	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design					
e	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent and solvent negative control groups.	
	Metric 5:	Negative Control Response	High	All solvent control animals were immobilized at 48hr (page 7/10). The treatment con- centration (10 mg/L DEHP) and control were both 0% immobilized.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	naracterization				
Domain D. Exposure er	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8:	Consistency of Exposure	Low	Not enough information reported to adequately assess this metric.	
		Administration	_		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration used with no effects reported.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	The test concentration was 10mg/L which is over the solubility reported in the final Scope for DEHP (0.2 mg/L).	
Domain 4: Test Organia	m				
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of the test animals was not reported	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
		Conditions			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to maintenance of health.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
Continued on next page					

Diethylhexyl Phthalate

HERO ID: 1335360 Table: 2 of 2

		contin	ued from previ	ous page			
Study Citation:	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) ph	thalate to Chiro	nomus tentans.			
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	0 - 4 days (0-9	6h)			
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1335360	1335360					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported.			
Domain 6: Confounding	/ Variable Co	ntrol					
Domain of Comounding	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups			
		Design and Procedures	mgn	There were no reported amorpholog anong the study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	veic					
Domain 7. Data Present	Metric 21.	Statistical Methods	N/A	Only one concentration assessed			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
		r ····································	-8	· · · · · · · · · · · · · · · · · · ·			
Additional Comments:	This form is were used to	for the mortality outcome reported in the p calculate the 48-hour median effect concent	aper. Test conc ration. EC50 an	entrations and corresponding percent mortality data derived from definitive tests d 95% confidence intervals.			
		· · · ·					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Park, S. Y., C pollutant exp Overall Dura Aquatic (fres	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environment International 33(6):817-822. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa. Species. Age:	Invertebrate:	Arthropods: Chironomus tentans: Larvae				
Health Outcome:	Mortality	1				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	674438					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Medium	The chemical substance was identified as bis(2-ethylhexyl) phthalate, but no information on substance form or CASRN was provided.		
	Metric 2:	Test Substance Source	Low	The test substance source was not provided, and its identity was not analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.		
Domain 2: Test Design			_			
	Metric 4:	Negative Controls	Low	The authors reported using a negative control, but it was unclear whether the chemical was in a solvent or if a solvent control was utilized.		
	Metric 5:	Negative Control Response	Low	Results for negative controls were not provided.		
	Metric 6:	Randomized Allocation	Low	The study did not indicate randomization.		
Domain 3: Exposure Ch	aracterization					
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media Preparation	Low	The study did not detail test media preparation methods.		
	Metric 8:	Consistency of Exposure	High	Exposures were consistently administered across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not analytically measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was 24-hours.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Information on exposure concentrations and spacing of exposure groups was not pro- vided. However, some detail on a dose range is provided in Table 1.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were not provided.		
Domain 4: Test Organisr	n					
C	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and from a reliable source (Korea Insti- tute of Toxicology).		
	Metric 14:	Acclimatization and Pretreatment	Low	No acclimatization period was reported.		
	Metric 15:	Conditions Number of Organisms and	Low	Ten C. tentans larvae per concentration were exposed to each of four test concentrations,		
		Replicates per Group		and there was at least one control group. No replication was reported.		

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 674438 Table: 1 of 1

		conti	nued from p	revious page				
Study Citation:	Park, S. Y., O	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environment International 33(6):817-822						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route.	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:	1 、							
Taxa, Species, Age:	Invertebrate: Arthropods; Chironomus tentans; Larvae							
Health Outcome:	Mortality	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	674438							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Although adequate conditions were described prior to testing, details are lacking for the acute toxicity test solution.				
	Metric 17:	Outcome Assessment Methodology	Medium	Mortality was the endpoint of interest, but there were no details on how this was deter- mined in C. tentans (e.g. not moving for a certain period of time).				
	Metric 18:	Consistency of Outcome Assessment	High	Mortality was determined at 24 hr of exposure.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences among study groups unrelated to exposures.				
Domain 7: Data Presen	tation and Anal	vsis						
	Metric 21:	Statistical Methods	High	The study authors used probit analysis with confidence intervals to determine effect concentrations (EC10, EC50 and EC90).				
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented in Table 1, but control outcomes were not provided for comparison.				
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Confidence intervals for LC10, LC50, and LC90 values were presented in Table 1 (unclear how many replicate experiments were conducted).				
Additional Comments:	The mortalit	y endpoint in C. tentans received a low rati	ng due to lac	k of experimental details and limited presentation of the data.				

Overall Quality Determination

Diethylhexyl Phthalate

Low

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.					
Duration: Exposure Route, Media, Path:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake rout					
Taxa, Species, Age:	Invertebrate	; Arthropods; Chironomus tentans; Larvae				
Health Outcome:	Developmen	nt/Growth				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	679311					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	High	The chemical was identified as a by name. Further details such as CASRN were pro- vided in Call et al 2001.		
	Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).		
	Metric 3:	Test Substance Purity	High	The purity was identified as 99% in the cited reference Call et al 2001.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.		
	Metric 5:	Negative Control Response	High	The biological response of control group was appropriate as shown in Table 5.		
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and addi- tion of sediment to the test beakers were described in detail.		
	Metric 8:	Consistency of Exposure Administration	High	The exposure consistency was reported and consistent.		
	Metric 9:	Measurement of Test Substance Concentration	High	The concentrations were measured using HPLC as described in the methods, and they were referenced in Call et al 2001.		
	Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design and fol- lowed cited methods (EPA, 1994).		
	Metric 11:	Number of Exposure Groups/	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.		
Domain 4: Test Organis	sm					
5	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of the test organisms prior to exposure was not reported.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per		
		Replicates per Group		beaker and two silica sand control replicates with 10 test organisms per beaker.		

		conti	nued from p	revious page			
Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815						
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	l0 days				
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	1		5				
Taxa, Species, Age:	Invertebrate:	Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Development/Growth						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	679311						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity on days 1 and 9.			
	Metric 17:	Outcome Assessment Methodology	High	The sediment was sieved, and survivors were collected, dried, and weighed.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no reported differences among study groups that could influence outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were re- ported for each study group, and there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	veis					
Domain (). Data Prosent	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman–Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dun- nett'sprocedure using a SigmaStatt Program.			
	Metric 22:	Reporting of Data	High	Treatment and control data were reported in Table 5. Results were represented as the average dry weight per individual.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.			
Additional Comments:	None						
Overall Quality Determination			High				

HERO ID: 679311 Table: 2 of 2

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stu	idy authors (i	.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	• •	··· · ·					
Taxa, Species, Age:	Invertebrate;	; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	679311						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. Further details such as CASRN were provided in Call et al 2001.			
	Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).			
	Metric 3:	Test Substance Purity	High	The purity was identified as 99% in the cited reference Call et al 2001.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.			
	Metric 5:	Negative Control Response	High	The biological response of the control group was appropriate as shown in Table 5.			
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and the addition of sediment to test beakers were described in detail.			
	Metric 8:	Consistency of Exposure	High	Exposure consistency was reported and consistent.			
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods, and they were referenced in Call et al 2001.			
	Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed cited methods (EPA, 1994).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of the test organisms prior to exposure was not reported.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.			

Domain 5: Outcome Assessment

		conti	nued from p	revious page			
Study Citation:	Call, D. J., C Parkerton, T exposures F	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805–1815.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Chironomus tentans; Larvae						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	679311						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity on days 1 and 9.			
	Metric 17:	Outcome Assessment Methodology	Medium	Survivor count was determined after the 10 day exposure, but it was not reported as percent mortality.			
	Metric 18:	Consistency of Outcome	High	The outcome was assessed at the conclusion of the 10-day exposure.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among study groups that could influence outcome			
	Matria 20.	Design and Procedures	II: -1-	assessment.			
	Metric 20:	Outcomes Onrelated to Exposure	nıgıı	ported for each study group, and there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Procont	ation and Anal	voie					
Domain 7. Data Fresent	Metric 21.	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman-Karber			
	Methe 21.	Statistical Methods	mgn	method. Dry weight datawere analyzed by one-way analysis of variance and Dun- nett'sprocedure using a SigmaStatt Program.			
	Metric 22:	Reporting of Data	Medium	Data were reported in Table 5, however, results were pooled among replicates.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.			
Additional Comments:	None						
Overall Quality Determination		High					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Call, D. J., F., Reiley, N	Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., Vandeventer, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures.					
Duration	Overall Dur	Environmental Toxicology and Chemistry 20(8):1/98-1804.					
Fynosure Route	Aquatic (fre	shwater): Water: Not determined by study	authors (i e	chemical of interest in exposure water, but unable to determine exact untake route)			
Media. Path:	riquatie (iie	silvater), water, not determined by study	autions (i.e.,	enclinear of interest in exposure water, but anable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate	Invertebrate: Arthropods: Chironomus tentans: Larvae					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	679312						
Domain		Metric	Rating	Comments			
Domain 1: Test Substa	nce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, but no CASRN or structure were provided.			
	Metric 2:	Test Substance Source	High	The source of the phthalate was Aldrich Chemical. The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >98%.			
Domain 2: Test Design	1						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure C	haracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.			
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.			
Domain 4: Test Organi	ism						
_ shaan ii tost organi	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects.			

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Call, D. J., I F., Reiley, N	Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., Vandeventer, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures.					
Duration	Overall Dur	Environmental Toxicology and Chemistry $20(\delta)$: 1796-1804. Overall Duration: $A = 10$ days: Exposure Duration: $A = 10$ days					
Duranon. Exposure Doute	Aquatic (free	shuater): Water: Not determined by study	nuthors (i.e.	chamical of interact in exposure water, but unable to determine exact untake route)			
Media, Path:	Aquate (neshwater), water, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	679312						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions of the test system were conducive to the maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.			
	Metric 18:	Consistency of Outcome	High	The outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.			
Additional Comments:	None						
Overall Quality Determination		High					

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Study Citation:	Adams, W. J organisms. F	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.						
Duration: Exposure Route, Modia Path:	Overall Dura Aquatic (free	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Immobilizati Di-ethylhexy 1321996	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Immobilization Di-ethylhexyl phthalate (DEHP) 1321996						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		_					
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low Low	Test substance nomenclature was reported without a CASRN. The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No				
	Metric 3:	Test Substance Purity	High	analytical data was reported. At least 95% purity was reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5:	Negative Control Response	High	The control response was acceptable.				
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or the measures taken to prevent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.				
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.				
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.				
Domain 5: Outcome Ass	sessment							

Diethylhexyl Phthalate

HERO ID: 1321996 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Adams, W. J organisms, F	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Not Applica	able (e.g., fur	ngi or algae studies) or Not Reported			
Health Outcome:	Immobilizat	ion					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.			
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent across groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.			
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Quali	Overall Quality Determination		High				

Study Citation:	Bionomics,	Bionomics, Springborn (1984). Acute toxicity of fourteen phinatate esters to Daphnia magna (final report).					
Duration:	Overall Dura	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	(I phthalate (DEHP)					
HERO ID:	1316223						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Medium	The chemical is identified by name and CASRN. No other verification is provided.			
	Metric 2:	Test Substance Source	Low	Chemicals were provided by the General Electric Company in 1-L amber glass bottles. No analytical verification or additional information was provided.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.			
Domain 2: Test Design	36.1.4						
	Metric 4:	Negative Controls	Hıgh	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).			
	Metric 5:	Negative Control Response	High	No mortality or adverse effects were reported in the controls.			
	Metric 6:	Randomized Allocation	Medium	Test organisms were impartially distributed among test containers.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately re- ported but did not account for physical-chemical properties, specifically the low solubil- ity in the test medium and rapid degradation rate. The test concentrations were very low at the end of the test, but the authors reported all of the concentrations. Any endpoint should be calculated in terms of the mean-measured concentration rather than the initial measured concentration, which is reported in the study.			
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods. Analytical technologies used were highly sensitive (GC-MS).			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (acute Daphnia study of 48-hour duration).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Test concentrations were spaced with the intention of bracketing the limit of solubility, but the actual measured test concentrations were very low. The results of this test can only be used to characterize the toxicity up to the limit of solubility of the test medium, which may or may not reflect the solubility reported in other sources.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The authors reported that the initial test featured a film that captured and killed a sig- nificant number of the daphnia. A solvent should have been used. As this did not cause excess mortality in the test, this was not determined to negatively affect the results of the test.			

Domain 4: Test Organism

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to Daphnia magna (final report). **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile **Health Outcome:** Mortality Di-ethylhexyl phthalate (DEHP) Chemical: HERO ID: 1316223

Domain	Metric	Rating	Comments
Metric 13:	Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups. As excess mortality was not observed in the controls, this was not determined to adversely affect the out- come.
Metric 15:	Number of Organisms and Replicates per Group	Low	5 daphnia/replicate is far lower than the guideline required 20 daphnia/replicate.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and the biomass loading was appropriate.
Metric 17:	Outcome Assessment Methodology	Low	Significant deficiencies in the reported outcome assessment methodology were identi- fied. The test was intended to quantify the mortality up to the limit of solubility of the chemical. This was not accomplished due to the extremely low solubility in the test medium. In both the definitive and the corroborative tests, the authors report that a film of insoluble test material formed on the surface that entrapped some of the test organ- isms but didn't result in mortality in the corroborative test.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Co	ontrol		
Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
Noule 17.	Design and Procedures	mgn	or other factors that could influence the outcome assessment.
Metric 20:	Outcomes Unrelated to Exposure	Low	A film of insoluble test material may have affected the test organisms.
Domain 7: Data Presentation and An	alvsis		
Metric 21:	Statistical Methods	N/A	No mortality was observed, so statistical analysis was not needed.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest.
Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not conduct a solubility test in the test medium that would have ex- plained why the measured test concentrations were so low in comparison to the reported solubility of the test material elsewhere.

Diethylhexyl Phthalate

		. continued from previous page			
Study Citation:	Bionomics,, Springborn (1984). Acute toxicity o	f fourteen phthalate esters to Daphnia	a magna (final report).		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure I	Ouration: 0 - 4 days (0-96h)			
Exposure Route,	Aquatic (freshwater); Water; Not determined by	study authors (i.e., chemical of intere	st in exposure water, but unable to determine exact uptake route)		
Media, Path:					
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juver	nile			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1316223				
Domain	Metric	Rating	Comments		
Additional Comments:	This study result should be interpreted with cau	ation. This test was comprised of tw	vo parts- a definitive and corroborative test. The definitive test was		
	not valid because a film of insoluble test material formed on the surface of the test medium, causing excess mortality. In the corroborative test, the				
	material apparently also formed a film, but no mortality was observed. Results should be calculated in terms of mean-measured test concentration of				
	the concentrations at the beginning and end of the tests, rather than only at the initiation of the test, which is how the authors report the study results.				
	Furthermore, no solubility test was conducted with	th the test medium, so the results may	y not be representative of the solubility of DEHP in a natural aquatic		
	system.				

Overall Quality Determination

Medium

Study Citation:	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.						
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	ition: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study a	n: 0 - 4 days (0-96h) authors (i.e., chemical of inte	rest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	679904						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by nomenclature (Table 1a).			
	Metric 2:	Test Substance Source	High	The sources of the test substances were identified (Table 1a).			
	Metric 3:	Test Substance Purity	High	"These samples are commercial products with stated purities in excess of 99.5 (w/w)."			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	"Five Daphnia were used in each test beaker with two replicates for the control, the dispersant control (10 mg/l) and for each phthalate treatment."			
	Metric 5:	Negative Control Response	High	No effects were observed in any controls.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	A large amount of surfactant (10x the concentration of phthalate) was used to ensure that the phthalate was suspended in the test solution.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	Uninformative	Exposure concentrations were not verified in the acute tests, and due to the use of sur- factant to attain nominal concentrations well in excess of solubility ("three orders of magnitude" according to the paper), there is serious doubt that actual concentrations re- semble nominal. Although the authors report that for the chronic tests the actual concen- trations resembled nominal when tested in freshly prepared treatment solution, they note that "phase separation of the dispersions had occurred in storage and the dispersions were thoroughly stirred before analysis". This does not resemble exposure conditions.			
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency were appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	This is a limit test ("3 orders of magnitude" above solubility limit, no adverse effects			
		Spacing of Exposure Levels		noted in any exposure).			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations exceeded the solubility limit but were within the dispersibility limit with the dispersants used, as noted by the authors.			
Domain 4: Test Organis	m						
en e	Metric 13:	Test Organism Characteristics	High	"The test organism was the freshwater crustacean Daphnia magna Strauss, derived from continuous laboratory cultures."			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	"The cultures were maintained under similar conditions (water, temperature, photope- riod and feeding) to those described for the reproduction test"			
			Continued on next page				

Diethylhexyl Phthalate

		con	tinued from previous	s page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 679904				
Domain		Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	N/A	This is a limit test.	
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ""The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."	
	Metric 17:	Outcome Assessment Methodology	High	Mortality was evaluated as immobilization.	
	Metric 18:	Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding	y / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None				

Overall Quality Determination

Uninformative

Study Citation:	Brown, D., 7	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426				
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate:	Invertebrate: Arthropods: Daphnia magna: Iuvenile				
Health Outcome:	Immobilization					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	1334281	() primi (())				
Domain	Metric Rating Comments					
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
-	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	paracterization					
Domain 5. Exposure er	Metric 7	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-		
	Mettre 7.	Preparation	Wiedrum	ported.		
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered		
		Administration	6	consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and were similar to nominal concentrations.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4: Test Organis	m					
Domain 4. Test Organis	Matria 13	Test Organism Characteristics	Madium	There are minor reconvisions or uncortainties about the test species source		
	Metric 13.	A colimatization and Protrootmont	Low	The study did not report whether test enconisms were exclimatized.		
	Metric 14:	Conditions	LOW	The study and not report whether test organisms were accumatized.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
Domain 5: Outcome As	sessment		TT' 1			
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.		
	Continued on next page					

		contin	ued from previo	us page			
Study Citation:	Brown, D., ' the reproduc	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Immobilization						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1334281						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Medium	Details regarding the execution of the study protocol for outcome assessment were lim-			
		Assessment		ited.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.			
Domain 7: Data Present	ation and Anal	lvsis					
	Metric 21:	Statistical Methods	Low	Though statistical analysis was not conducted, there was no effect seen at any concentra- tion.			
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	Medium				

Study Citation:	Huang, B., Li, D., Yang, Y. (2016). Joint toxicity of two phthalates with waterborne copper to Daphnia magna and Photobacterium phosphoreum. Bulletin					
Duration:	of Environm Overall Dura	ental Contamination and Toxicology 97(3 ation: 0 - 4 days (0-96h); Exposure Duration):380-386. on: 0 - 4 days	(0-96h)		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	T . 1 .					
Taxa, Species, Age:	Invertebrate;	Arthropods; <i>Daphnia magna</i> ; Juvenile				
Chemical:	Di-ethylbey	ION 1 phthalate (DEHP)				
HERO ID:	5750702	(DEIII)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source was reported, but the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Medium	It was mentioned that the chemicals used were analytical grade.		
Domain 2: Test Design						
6	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7	Experimental System/Test Media	Medium	Test media preparation methods were reported but did not provide the measures taken to		
	Wette 7.	Preparation	Wiedium	minimize loss of test substance before and during the exposure. The concentration of the test substance was measured during the study.		
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using HPLC. The percent recovery of spiked test substance was within an acceptable range. Measured concentrations were similar to nominal concentrations.		
	Metric 10:	Exposure Duration and Frequency	Medium	Minor limitations in exposure frequency and duration of exposure were identified -acute daphnid toxicity study of 24-hour duration as opposed to 48 hours.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	7 exposure groups and a control were tested.		
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate. No effects on biological responses were observed in the solvent control.		
Domain 4: Test Organisi	n					
c c	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 daphnids per test vessel, and they were tested in triplicate.		

Diethylhexyl Phthalate

		contir	nued from p	revious page				
Study Citation:	Huang, B., L	i, D., Yang, Y. (2016). Joint toxicity of two ental Contamination and Toxicology 97(3):	phthalates v	vith waterborne copper to Daphnia magna and Photobacterium phosphoreum. Bulletin				
Duration:	Overall Dura	tion: 0 - 4 days (0-96h): Exposure Duration	n: 0 - 4 davs	(0-96h)				
Exposure Route.	Aquatic (fres	hwater): Water: Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	\mathbf{r}							
Taxa, Species, Age:	Invertebrate:	Invertebrate: Arthropods: Daphnia magna: Juvenile						
Health Outcome:	Immobilizati	on						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	5750702							
Domain		Metric	Rating	Comments				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food and biomass loading seem to be conducive to the maintenance of health. The authors followed the protocol outlined in the the National StandardMethod of China (GB/T 13266-1991).				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome (immobilization of daphnids) assessment methodology was not clearly reported.				
	Metric 18:	Consistency of Outcome Assessment	High	Immobilization was recorded after 24 hours of exposure in all treatment groups and the control.				
Domain 6: Confounding	/ Variable Cor	ıtrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. The methods for calculating effect ratio (y-axis of Fig 1) and EC 50 values were not provided.				
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group via figures. The methods for calculating effect ratio (y-axis of Fig 1) were not provided. EC 50 values were given without confidence intervals in the text.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	Data analysis	Data analysis methods were not provided. The EC 50 value was given without confidence intervals.						

Overall Quality Determination

High

Study Citation:	Jonsson, S.,	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology						
Duration: Exposure Route, Media, Path:	and Chemist Overall Dura Aquatic (free	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Larvae						
Health Outcome:	Immobilizat	immobilization						
Chemical: HERO ID:	D1-ethylhexy 789536	Di-ethylhexyl phthalate (DEHP) 789536						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively. Nomenclature, CASRN, and structure were reported.				
	Metric 2:	Test Substance Source	High	DEHP was purchased from Merck Eurolab (Stockholm, Sweden), but the test substance identity was NOT analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Percent purity was reported as >98%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	Low	The biological response of the control group was not clearly reported. It was stated that tests were repeated if the mortality in the control group exceeded 5%.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of test substance before and during the expo- sure.				
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were measured but not reported.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the exposure concentrations and the spacing of exposure levels.				
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per concentration with 5 animals used in each replicate.				
		(Continued on next page .					

Diethylhexyl Phthalate

HERO ID: 789536 Table: 1 of 1

		cont	tinued from previou	s page			
Study Citation:	Jonsson, S., and Chemist	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration: 0	- 4 days (0-96h)				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	-						
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Larvae						
Health Outcome:	Immobilizat	Immobilization					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	789536						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate and whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	EC10 and EC 50 values were calculated using probit analysis with maximum likelihood estimation.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained. Authors noted surface entrapment of test animals in the highest tested concentration, but these were excluded from the calculation of EC 10 and EC 50 values.			

Additional Comments: The exposure concentrations, spacing of exposure levels, and control response were not reported. Measured concentrations were not reported. Mortality data were not provided for each of the treatment groups and control. Only 24 and 48 hour EC 10 and EC 50 values were reported.

Overall Quality Determination

Uninformative

Study Citation:	Jordão, R., O	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.							
Duration	Science of the	he Total Environment 545-546(Elsevier):12	7-136. n: 0 - 4 days (0 0						
Duration: Evnosuro Douto	Aquatic (free	ation: 0 - 4 days (0-901); Exposure Duration	1: 0 - 4 days (0-9)	1011) nical of interact in exposure water, but unable to determine exact untake route)					
Madia Dath.	Aquatic (fre	(nvertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile Behavioral							
Tava Spacias Aga	Invertebrate								
Health Outcome	Behavioral								
Chemical.	Di-ethylbey	vl phthalate (DFHP)							
HERO ID:	3070913								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce		8						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.					
	Metric 2:	Test Substance Source	High	"Optimized LC and MS/MS parameters for each analyte were based on reported infor-					
			8	mation and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."					
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.					
Domain 2: Test Design	Matric 4.	Negative Controls	High	Study authors reported using an appropriate consurrant column control group					
	Metric 5:	Negative Control Pesponse	High	The biological response of the negative control groups was reported					
	Metric 5:	Pandomized Allocation	Low	Passarahara did nat rapart haw arganisms wara allogated to study groups					
	Metric 0.	Kandomized Anocation	LOW	Researchers and not report now organisms were anocated to study groups.					
Domain 3: Exposure Ch	naracterization								
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare					
		Preparation		test concentrations.					
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions may have an impact on results.					
		Administration							
	Metric 9:	Measurement of Test Substance	Medium	The high concentration was measured as fresh and aged but not as the actual experimen-					
		Concentration		tal doses.					
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.					
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were justified for a					
	14	Spacing of Exposure Levels	TT: 1	dose response.					
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.					
Domain 4: Test Organis	m								
2 chian ii rost organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source					
	Metric 14	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized					
	incure i li	Conditions	2011	The stacy and not report whether tost organisms were deeminuted.					
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to char-					
		Replicates per Group		acterize toxicological effect.					

		contin	ued from previo	ous page				
Study Citation:	Jordão, R., C	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.						
D (1	Science of th	Science of the Total Environment 545-546(Elsevier):127-136.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-9)	6h)				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
Taxa, Species, Age:	Invertebrate;	; Arthropods; Daphnia magna; clone F; Juve	enile					
Health Outcome:	Behavioral							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	3070913							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.				
	Metric 17:	Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.				
Domain 6: Confounding	y / Variable Co	ntrol						
Domain of Companying	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Some of the statistical analysis methods were described in Section 2.7 (page 5/10), but methods on EC estimation methods were not reported.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	The behavio	ral outcome is to account for feeding inhibiti	ion.					
Overall Quali	ty Deterr	nination	Medium					

Study Citation:	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.					
	Science of the Total Environment 545-546(Elsevier):127-136.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (reshwater); water, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	Investigation of Anthropological and the Investigation of the Investigat					
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; clone F; Juvenile					
Health Outcome:	Development/Glowin Di athylhavyl phthalata (DEHD)					
UEDO ID.						
HERO ID:	3070913					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
	Metric 2:	Test Substance Source	High	"Optimized LC and MS/MS parameters for each analyte were based on reported infor- mation and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."		
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions may have an impact on results.		
	Metric 9:	Measurement of Test Substance	Medium	The high concentration was measured as fresh and aged but not as actual experimental doses.		
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	M (15	Conditions				
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to char-		
		Replicates per Group		מנורובע מ וטאונטוטפונמו בוובנו.		
Domain 5: Outcome Ass	sessment					
20man 9. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	The environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.		
		Conti	nued on next pa	ge		

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Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Jordão, R., C	Garreta, E., Campos, B., Lemos, M. F., Soare	s, V.,M, A.M., T	auler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.			
	Science of th	e Total Environment 545-546(Elsevier):127-	136.				
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-96	h)			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; clone F; Juvenile					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3070913	3070913					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported.			
Domain 6: Confounding	/ Variable Cor	ntrol					
C	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.			
Domain 7: Data Presenta	ation and Anal	vsis					
Domain 7. Data Presenta	Metric 21.	Statistical Methods	Low	Statistical analysis was assumed to be performed, but it was not described in the statisti-			
	Methe 21.	Statistical Methods	Low	cal analysis section (2.7 page 5/10).			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluati	on was for body length from Table S3.					
Overall Quality Determination Medium							

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Study Citation:	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.					
	Science of the Total Environment 545-546(Elsevier):127-136.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (fres	shwater); Water; Not determined by study a	uthors (i.e., chem	ical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; clone F; Juv	enile			
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	3070913					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
	Metric 2:	Test Substance Source	High	"Optimized LC and MS/MS parameters for each analyte were based on reported infor- mation and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."		
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.		
Domain 2: Test Design	Matria 4.	Nearting Controls	II:-1			
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported for molts.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization		Ţ			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Medium	Reporting omissions may have an impact on results.		
	Metric 9:	Measurement of Test Substance	Medium	The high concentration was measured as fresh and aged but not as the actual experimen- tal doses.		
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was marked medium based on uncertainties in exposure duration which was		
	M (11		т	based on developmental stage.		
	Metric 11:	Number of Exposure Groups/	Low	The number or range of concentrations was not reported.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented the determination of whether exposure concentrations exceeded the water solubility limit.		
Domain 4: Test Organisi	m		TT' 1			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to char- acterize a toxicological effect.		
		· · ·				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.		
Continued on next page						

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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 3070913 Table: 3 of 5

		contif	nued from previ	ous page		
Study Citation:	Jordão, R., C	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna. Science of the Total Environment 545-546(Elsevier):127-136				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; clone F; Juvenile				
Health Outcome:	Development/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3070913					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not		
		Assessment		reported.		
Domain 6: Confounding	g / Variable Co	ntrol				
c	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental condition		
		Design and Procedures	C			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical estimation methods of the molt LOEC concentration were not reported.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group but results were described in the text.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion was for molt.				

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Study Citation:	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.						
D	Science of the Total Environment 545-546(Elsevier):127-136.						
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	n: $0 - 4 \text{ days} (0-9)$	6h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chem	incal of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	T (1 (.1				
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; clone F; Juve	enile				
Chamicala	Di athulhau	d abthalata (DEUD)					
HFRO ID.							
Domain	Metric Rating Comments						
Domain 1: Test Substand	Ce Matria 1.	Test Substance Identity	Uiah	The sharring laws identified have and CACH			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	High	"Optimized LC and MS/MS parameters for each analyte were based on reported infor- mation and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves"			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.			
Domain 2: Test Design			TT: 1				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported for mortality.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		Ŧ				
	Metric /:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions may have an impact on results.			
	Metric 9:	Measurement of Test Substance	Medium	The high concentration equivalent was measured as fresh and aged but not as actual experimental doses.			
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration, which was based on developmental stage.			
	Metric 11:	Number of Exposure Groups/	Low	The number or range of concentrations was not reported.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit.			
Domain 4: Test Organisi	m		TT' 1	man and the second s			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to char- acterize toxicological effect.			
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.			
Continued on next page							

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Diethylhexyl Phthalate

		conti	nued from previ	ous page			
Study Citation:	Jordão, R., C Science of th	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna. Science of the Total Environment 545-546(Elsevier):127-136.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Arthropods; Daphnia magna; clone F; Juv	enile				
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3070913	-					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Medium	These are minor uncertainties that are unlikely to have a substantial impact on results.			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported.			
Domain 6: Confoundir	og / Variable Co	ntrol					
Domain 0. Comoundin	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	methe 19.	Design and Procedures	mgn	There were no reported differences among the study groups in environmental conditions			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.			
Domain 7: Data Preser	ntation and Anal	lysis					
	Metric 21:	Statistical Methods	Low	No statistical analysis on mortality was reported.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group but results were described in the text.			
	Matria 23.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			

Medium

Overall Quality Determination

Page **433** of **958**

Study Citation:	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.					
	Science of the Total Environment 545-546(Elsevier):127-136.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chem	nical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; clone F; Juv	enile			
Health Outcome:	Nutritional &	ż Metabolic				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	3070913					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		-			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
	Metric 2:	Test Substance Source	High	"Optimized LC and MS/MS parameters for each analyte were based on reported infor-		
			6	mation and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."		
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 2. Europuna Ch	anatonization					
Domain 5: Exposure Ch	Motrio 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to engennicitally propose		
	Metric 7:	Preparation	Low	test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Medium	Reporting omissions may have an impact on results.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	The high concentration was measured as fresh and aged but not as the actual experimen- tal doses.		
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.		
			6			
Domain 4: Test Organis	m					
C C	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
		Conditions				
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to char-		
		Replicates per Group		acterize toxicological effect.		
Domain 5: Outcome Ass	sessment		Ŧ			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.		
		Conti	nued on next pa	ge		

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Diethylhexyl Phthalate

		continu	ued from previ	ous page			
Study Citation:	Jordão, R., C	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in Daphnia magna.					
T	Science of th	ne Total Environment 545-546(Elsevier):127-	-136.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-9	6h)			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; clone F; Juvenile					
Health Outcome:	Nutritional &	Nutritional & Metabolic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3070913						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported.			
Domain 6: Confounding	y / Variable Cor	ntrol					
c	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	e				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.			
Domain 7: Data Present	ation and Anal	veis					
Domain 7. Data Present	Matria 21:	Statistical Mathada	Low	Some of the statistical analysis matheda ware described in Section 2.7 (mag. 5/10), but			
	Methic 21.	Statistical Methods	LOW	methods on EC estimation were not reported.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Nile red fluo	rescence was used to determine lipid content	. Data/results w	vere presented in Table 2, Page 6/10.			
Overall Qualit	ty Detern	nination	Medium				

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Study Citation: Duration:	Monsanto, (1 Overall Dura	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (fres	hwater); Water; Dermal (topical application	on)				
Media, Path: Taxa, Species, Age:	Invertebrate:	Arthropods: Daphnia magna: Juvenile					
Health Outcome:	Mortality	nunopous, Dupinna magna, su tenne					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1335345						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name. No CASRN or structure were reported.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control and the solvent control were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
Bollium 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations.			
	Metric 8:	Consistency of Exposure	Hıgh	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	All test concentrations have exceeded solubility, but a solvent was used to aid solubility. The lowest concentration in the bioassay was 1 mg/L, and the solubility listed in the Final Scope for DEHP is 0.27 mg/L.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
		· F · · · · · · · · · · · · · · · · · ·					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.			
Continued on next page							

Diethylhexyl Phthalate

		contin	ued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Monsanto, (1 Overall Dura Aquatic (fres Invertebrate; Mortality Di-ethylhexy 1335345	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Dermal (topical application) Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 1335345				
Domain		Metric	Rating	Comments		
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome	High High	The outcome assessment methodology addressed the intended outcome of interest. Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	/ Variable Cor Metric 19: Metric 20:	Assessment trol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High High	There were no reported differences among the study groups in environmental conditions. There were no differences among groups.		
Domain 7: Data Presenta	ation and Analy Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Statistical methods were adequately described. Data for exposure-related findings were presented for each treatment and control group. There were no unexpected outcomes.		
Additional Comments: None Overall Quality Determination High						

Study Citation: Duration: Exposure Route, Media Path:	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna in the presence of fulvic acid. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1335353					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT' 1			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
	Metric 2:	Test Substance Source	Low	verified by the performing laboratory."DEHP (Lot-#QL-1000), a clear liquid, was ob- tained from Robert H. Mills (MIC) and was used as the test chemical."		
	Metric 3:	Test Substance Purity	Low	The purity or grade of the test substance were not reported.		
Domain 2: Test Design			TT' 1			
	Metric 4:	Negative Controls	High	study authors reported using an appropriate concurrent negative control group 'consist- ing of the same dilution water and conditions but with no test compound or ethanol," as well as a positive control "consisting of the same dilution water, conditions, and ethanol."		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 2: Exposure Ch	areatarization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit; ethanol was used to aid solubility.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
		Cont	inued on nex	t page		

Diethylhexyl Phthalate

		contir	nued from p	previous page		
Study Citation: Duration: Exposure Route, Media, Path:	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to Daphnia magna in the presence of fulvic acid. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Mortality	Mortality				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1335353					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
	_					

Overall Quality Determination

High

Study Citation:	Muller (1983). Determination of the acute toxicity of di-2-ethylhexyl-phthlat (dehp) to the waterflea daphnia magna straus.					
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	on: $0 - 4$ days	(0-96h)		
Exposure Roule, Media Path:	Aquatic (fres	snwater); water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate:	Arthropods: Daphnia magna: Juvenile				
Health Outcome:	Immobilizati	on				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	11328251					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test chemical was identified by name, formula, and manufacturer.		
	Metric 2:	Test Substance Source	Low	The manufacturer of the test substance was BASF. No analytical verification information was given for the test substance.		
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A control group was used in the test.		
	Metric 5:	Negative Control Response	High	There was no adverse effect on mobilization of the daphnia magna in the control groups.		
	Metric 6:	Randomized Allocation	Low	The report does not state how daphnids were allocated to experimental units.		
	, .					
Domain 3: Exposure Ch	aracterization		TT' 1			
	Metric 7:	Preparation	High	stance. A serial dilution was prepared in the solvent to make the test concentrations. EC values were based off of nominal concentrations.		
	Metric 8:	Consistency of Exposure Administration	High	Each test vessel got 10uL of the respective test dilution. All test vessels contained 10mL of solution.		
	Metric 9:	Measurement of Test Substance	Low	Test concentration measurements were not reported.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The test was run for 48 hours which is the standard for acute Daphnia magna tests.		
	Metric 11:	Number of Exposure Groups/	High	The test concentrations used were sufficient to determine the desired EC values.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	A solvent was used because the test substance was not soluble in water. No adverse effects were shown in the results due to the solvent.		
Domain 4: Test Organis						
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The strain of Daphnia magna used were originally obtained from a research institute in France and then bred in the ecology lab at BASF. Breeding conditions reported were adequate. Less than 24-hour old neonates were used in the test, which is according to standards.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Lighting, temperature, pH, and water hardness were all reported for lab conditions for daphnid cultures and the bioassay.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were five daphnia per test vessel and four replicates per test concentration.		

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Diethylhexyl Phthalate

Muller (1983). Determination of the acute toxicity of di-2-ethylhexyl-phthlat (dehp) to the waterflea daphnia magna straus. **Study Citation: Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile **Health Outcome:** Immobilization Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 11328251 Domain Metric Rating Comments Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High Breeding conditions prior to the test were reported and appropriate. Test conditions were reported and appropriate. Water chemistry measurements for the test are shown in Tables 1, 2, and 3. Metric 17: Outcome Assessment Methodology High Daphnia magna were assessed at zero, 24, and 48 hours for inability to swim after gentle agitation of the test vessels. These data were used to determine the EC values. Consistency of Outcome Metric 18: High The assessment of Daphnia magna mobility at the desired timepoints was done consistently across all test groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High Temperature, pH, and oxygen concentrations were reported across all test concentra-Design and Procedures tions. Metric 20: Outcomes Unrelated to Exposure High There was no information to suggest differences in test organisms among concentration groups that could influence the outcome assessment. There was no reason to suggest anything other than exposure concentrations were different among experimental units. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were not described but EC values were calculated in the results. A 95% confidence interval was shown in the results section, but no value was indicated for it (blank lines next to it). These results are shown on page 8 of the pdf. Standard LC50 calculations were performed. Very little immobilization was observed across treatments. Thus, LC50s and CIs could not be calculated for "no effect." Metric 22: Reporting of Data Data for Daphnia magna able to swim after 0, 24, and 48 hours was shown in Table 4 for High all test concentrations (averaged over all replicates). Table 1A shows data for Daphnia magna able to swim for all replicates, across all investigated timepoints and test concentrations.

Additional Comments: This evaluation is for the assessment of immobilization in Daphnia magna neonates exposed to DEHP for 48 hours.

Explanation of Unexpected Outcomes

Overall Quality Determination

Metric 23:

High

High

There were no unexpected outcomes. DEHP did not affect daphnids.

Study Citation: Duration: Exposure Route, Media, Path:	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk fro pollutant exposure. Environment International 33(6):817-822. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route and the product of the screening of the risk from the screening of the					
Taxa, Species, Age:	Invertebrate;	Arthropods; <i>Daphnia magna</i> ; Juvenile				
Chemical	Di-ethylbey	ul phthalate (DEHP)				
HERO ID:	674438	(PERIO)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Medium	The chemical substance was identified as bis(2-ethylhexyl) phthalate, but no information on substance form or CASRN was provided.		
	Metric 2:	Test Substance Source	Low	The test substance source was not provided, and its identity was not analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	The authors did not clearly indicate use of a negative control in the acute toxicity test for D. magna.		
	Metric 5:	Negative Control Response	Low	Results for negative controls were not provided.		
	Metric 6:	Randomized Allocation	Low	The study did not indicate randomization.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study did not detail test media preparation methods.		
	Metric 8:	Consistency of Exposure	High	Exposures were consistently administered across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not analytically measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was 24-hours.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Information on exposure concentrations and the spacing of exposure groups was not provided. However, Table 1 provides some information on the range of DEHP concentrations utilized in the study.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were not provided. Use of a solvent was not reported.		
Domain 4: Test Organis	m					
-	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and from a reliable source (Korea Insti- tute of Toxicology).		
	Metric 14:	Acclimatization and Pretreatment	Low	No acclimation period was reported.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Ten D. magna were exposed per test concentration. No replication was reported.		

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 674438 Table: 1 of 1

		conti	nued from p	revious page				
Study Citation:	Park, S. Y., C	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environment International 33(6):817-822.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	(0-96h)				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media. Path:	1	\mathbf{I}						
Taxa. Species. Age:	Invertebrate:	Invertebrate; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Immobilizat	Immobilization						
Chemical:	Di-ethylhexy	Di-ethylhexyl nhthalate (DEHP)						
HERO ID:	674438							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Although adequate conditions were described prior to testing, details are lacking for the acute toxicity test solution.				
	Metric 17:	Outcome Assessment Methodology	Medium	Swimming inhibition was utilized as an endpoint. More detail could have been provided of what authors determined as inhibition.				
	Metric 18:	Consistency of Outcome Assessment	High	Swimming inhibition was determined after 24 hr of exposure.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences among study groups unrelated to exposures.				
Domain 7: Data Presen	tation and Anal	vsis						
Domain / Dua Presen	Metric 21:	Statistical Methods	High	The study authors used probit analysis with confidence intervals to determine effect concentrations (EC10, EC50 and EC90).				
	Metric 22:	Reporting of Data	Low	Effective concentrations for exposure-related findings were presented in Table 1, but control outcomes were not provided for comparison.				
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Confidence intervals for EC10, EC50, and EC90 values were presented in Table 1 (un-				

Overall Quality Determination

Low

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Scanlan, L. 1 K., Falciani, mechanisms Overall Dura Aquatic (fres Invertebrate; Mechanistic- Di-ethylhexy 2966135	 Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49(12):7400-7410. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Daphnia magna</i>; Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 2966135 					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Medium	The correct nomenclature and figure of the structure were reported. The compound was referred to as "bis(2-ethylhexyl) phthalate." This is a synonym for DEHP.			
	Metric 2:	Test Substance Source	Low	The source was listed as Aldrich with no analytical verification reported.			
	Metric 3:	Test Substance Purity	Low	No purity or grade were reported.			
Domain 2: Test Design	Metric 4:	Negative Controls	Low	The actual concentration of DMSO in the solvent control was not reported, but it was reported to be the same as the one used in the exposure concentration, which was reported to be 1/10 of the LC50 value.			
	Metric 5:	Negative Control Response	Low	The control values for the gene expression microarray were averaged and then used as reference values to create fold changes in specific genes in addition to authors use of housekeeping genes (actin and GAPDH) to normalize responses. However, the response of just the control was not reported.			
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.			
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	The study authors did not say explicitly what was done (e.g., flow through or static exposure). Exposure concentrations also were not measured. The material used for the exposures (e.g., glass, plastic) was not mentioned. Sorption out of the exposure water			
	Metric 8:	Consistency of Exposure	High	may have occurred. Exposures were administered consistently among treatments and controls.			
	Metric 9:	Administration Measurement of Test Substance	Low	Concentrations were reported as nominal (1/10 of LC50), and no analytical verification was performed.			
	Metric 10:	Exposure Duration and Frequency	High	The extraction of genes for a microarray could capture regulation of genes from an initial 48 hr exposure.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The micro array was conducted by exposing 15-20 adult Daphnids for 48 hour to a single treatment concentration (1/10th LC50).			
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentration was 1/10th the LC50. Authors indicated that 3.3 mg/L was the LC50 so 0.3 mg/L was the exposure concentration. The solubility from the Final Scope is listed at 0.27 mg/L.			

Environmental Hazard Evaluation

HERO ID: 2966135 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation: Duration:	Scanlan, L. K., Falciani, mechanisms Overall Dura	Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49(12):7400-7410. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome:	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Mechanistic Cell signaling/function						
Chemical: HERO ID:	Di-ethylhexy 2966135	yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The Daphnia were cultured asexually in a growth chamber and were acquired from ARO in New Hampshire. 14-d old daphnids were exposed in the study.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	The Daphina were maintained in COMBO media, and initial pH was reported. pH dur- ing the bioassay was not reported.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	It does not appear that the microarray portion of this study was replicated. The micro array was conducted by exposing 15-20 adult Daphnids for 48 hour to a single treatment concentration (1/10th LC50).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The culture media formula is listed in Table S1. Feeding protocols and water pH are described in the methods section.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods addressed the outcome of interest (gene expression compared to control conditions) and were sensitive enough to record data.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently applied across the one exposure and control treatment groups.			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among treatments and control to indicate that factors outside of the compound influenced the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors did not present information to suggest that animal health interfered with the outcome assessment.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	The analysis of the microarray was presented on page 3/11 in the "Gene ontology, path- way enrichment and cluster analysis" section.			
	Metric 22:	Reporting of Data	High	Figure 4 and table 1 (page 5/11) present the results of the microarray.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors indicated no unexpected outcomes.			
Additional Comments:	None						
Overall Oualit	t v Deter r	nination	High				

Study Citation:	Scanlan, L. I K., Falciani,	D., Loguinov, A. V., Teng, Q., Antczak, P. F., Stapleton, H. M., Vulpe, C. D. (2015).	, Dailey, K. P., N Gene transcriptio	Jowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. n, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse
Duration: Exposure Route, Media. Path:	mechanisms Overall Dura Aquatic (fres	of toxicity by legacy and emerging flame-re- tion: 0 - 4 days (0-96h); Exposure Duration hwater); Water; Not determined by study a	etardants. Enviro n: 0 - 4 days (0-9 uthors (i.e., chem	nmental Science & Technology 49(12):7400-7410. 6h) nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome:	Invertebrate; Mortality	Arthropods; <i>Daphnia magna</i> ; Larvae		
HERO ID:	2966135	I phinaiale (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Medium	Correct nomenclature and figure of the structure were reported. The compound is re- ferred to as "bis(2-ethylhexyl) phthalate" This is a synonym for DEHP.
	Metric 2:	Test Substance Source	Low	The source was listed as Aldrich with no analytical verification reported.
	Metric 3:	Test Substance Purity	Low	No purity or grade were reported.
Domain 2: Test Design	Matria 4.	Negative Controls	Low	
	Metric 4:	Negative Controls	Low	solvent controls were reported to be 0.05-0.1% DMSO. There were uncertainties re- garding the concentration of DMSO used in different DEHP exposures, or how much was used in the DMSO control exposure group.
	Metric 5:	Negative Control Response	High	The control response is reported in Table S2 of the Supplemental data as 5 organisms responding out of 135 total organisms.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
	, . <u>,</u> .			
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	Authors cited EPA Whole Effluent Toxicity guidelines, which include both static and flow-through experimental designs. The study authors did not say explicitly what was done. Exposure concentrations also were not measured. The material used for the exposures (e.g., glass, plastic) was not mentioned. Sorption out of the exposure water may have occurred.
	Metric 8:	Consistency of Exposure Administration	Medium	Concentrations of DMSO ranged between 0.05-0.1%. There were uncertainties regard- ing the concentration of DMSO used in the DMSO-control.
	Metric 9:	Measurement of Test Substance Concentration	Low	Concentrations were reported as nominal, and no analytical verification was performed.
	Metric 10:	Exposure Duration and Frequency	High	The 48 hr exposure duration for an acute bioassay with Daphnia is appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	According to supplemental table S2, the authors had 9 DEHP concentrations (0.3125-40 mg/L) and a DMSO control.
	Metric 12:	Testing at or Below Solubility Limit	Medium	All of the exposure concentrations (0-40 mg DEHP/L) for this bioassay were above the solubility listed in the Final Scope as 0.27 mg/L. The treatment concentrations are listed in the supplemental Table S2. The lowest treatment concentration is 0.3125 mg/L. The authors reported using 0.05-0.1% DMSO for the various chemicals tested, which may allow for 50-100 mg DEHP/L to be bioavailable. However, the authors did not report how much DMSO specifically was used to prepare the DEHP exposures; therefore, there is some uncertainty regarding the amount of carrier solvent (DMSO) present in all of the exposure groups. The mortality observed in the control exposure group was acceptable despite the uncertainty regarding the amount of DMSO that was used (5/135 organisms).

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Environmental Hazard Evaluation

HERO ID: 2966135 Table: 2 of 2

		contin	ued from previ	ous page					
Study Citation: Duration:	 Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49(12):7400-7410. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) 								
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study at	thors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	-								
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Larvae							
Health Outcome: Chemical:	Mortality Di-ethylbey	vl phthalate (DEHP)							
HERO ID:	2966135								
Domain		Metric	Rating	Comments					
Domain 4: Test Organi	sm								
0	Metric 13:	Test Organism Characteristics	High	The Daphnia were cultured asexually in a growth chamber and were acquired from ARO in New Hampshire.					
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	The Daphina were maintained in COMBO media, and initial pH was reported. PH dur- ing the bioassay was not reported.					
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The authors conducted the bioassay "similar to US EPA Whole Effluent Toxicity guide- lines." They used 4 reps per treatment concentration. This compound had 9 treatment concentrations and a control. The actual number of animals per treatment concentration was presented in Table S2.					
Domain 5: Outcome A	aggement								
Domain 5. Outcome A	Metric 16:	Adequacy of Test Conditions	High	The culture media formula is listed in Table S1. Feeding protocols and water pH are described in the methods section.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods addressed the outcome of interest (mortality) and were sensitive enough to record data.					
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently applied across treatments and control groups.					
Domain 6: Confoundin	g / Variable Co	ntrol							
Domain of Comoundain	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among treatments and controls to indicate that fac- tors outside of the compound influenced the outcome assessment.					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors did not present information to suggest that animal health interfered with the outcome assessment.					
Domain 7: Data Presen	tation and Anal	lysis							
	Metric 21:	Statistical Methods	High	Authors indicated that probit analysis was used for the formation of a dose-response curve with this compound (table S5).					
	Metric 22:	Reporting of Data	High	Data on mortality from each treatment concentration are presented in Table S2, and the Probit results with LC50 and 95% CI are presented in Table S5.					
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors indicated no unexpected outcomes.					
Additional Comments:	None								
Auditional Comments:	INOILE								

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Diethylhexyl Phthalate

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator Daphnia magna indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49(12):7400-7410. **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Larvae **Health Outcome:** Mortality Di-ethylhexyl phthalate (DEHP) Chemical: HERO ID: 2966135

Domain	Metric	Rating	Comments
Overall Quality Dete	ermination	Medium	

Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates or	n development, r	eproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total	
Duration: Exposure Route, Media Path:	Environmen Overall Dura Aquatic (free	t 654:969-977. ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study a	n: 0 - 4 days (0-9 uthors (i.e., chen	6h) nical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Invertebrate:	Arthropods; Daphnia magna; Juvenile			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)			
HERO ID:	5043468				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.	
Domain 2: Test Design					
Domani 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
			<u> </u>		
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15:	Conditions Number of Organisms and	Medium	The number of organisms and replicates were suitable.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	Low	Details of environmental conditions of the test system were not reported	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
		Conti	nuad on novt no	an	
Continued on next page					

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Diethylhexyl Phthalate

			aca moni previo	us page
Study Citation:	Seyoum, A., Environmen	Pradhan, A. (2019). Effect of phthalates on t 654:969-977.	development, re	production, fat metabolism and lifespan in Daphnia magna. Science of the Total
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-96	h)
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chemi	cal of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	_			-
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	5043468			
Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This was a 2	4 hr exposure that was measured at 96 hr.		
Overall Qualit	ty Detern	nination	Medium	

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E	7	(54.060.077	i development, r	eproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total				
Duration: C Exposure Route, A Media, Path:	Aquatic (fres	654:969-977. tion: > 21 days; Exposure Duration: 0 - 4 c hwater); Water; Not determined by study at	days (0-96h) uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: In	nvertebrate; Arthropods; Daphnia magna; Juvenile							
Health Outcome: R	Reproductive	/Teratogenic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID: 5	5043468							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance								
N	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.				
N	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
N	Metric 3:	Test Substance Purity	High	Chemical purity was reported as $>99\%$.				
Domain 2: Test Design								
U N	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
Ν	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.				
Ν	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Damain 2. Enname Cham								
Domain 3: Exposure Chara	acterization	Environmental Sectors /Teach Madia	I					
IV	Metric /:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare				
N	Metric 8.	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results				
1	vicule 0.	Administration	Wiedrum	Reporting onitissions are uninkery to have a substantial impact on results.				
Ν	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
Ν	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.				
Ν	Metric 11:	Number of Exposure Groups/	Low	Only one exposure group was reported.				
		Spacing of Exposure Levels						
Ν	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.				
Domain 4: Test Organism								
N	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
Ν	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
Ν	Metric 15:	Conditions Number of Organisms and	Medium	The number of organisms and replicates were suitable.				
		Replicates per Group						
Domain 5: Outcome Assess	sment							
N	Metric 16.	Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported				
N	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome				
N	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-				
1		Assessment	2011	ited.				

Domain 6: Confounding / Variable Control

Diethylhexyl Phthalate

HERO ID: 5043468 Table: 2 of 5

Study Citation:	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total				
Dunations	Environmen	t 654:969-977.	$d_{a} = (0, 0, 0, 0)$		
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: 0 - 4	days (0-96n)		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e., chemi	cal of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile			
Health Outcome:	Reproductive/Teratogenic				
Chemical:	Di-ethylbexyl phthalate (DEHP)				
HERO ID:	5043468				
			D. d		
Domain		Metric	Rating	Comments	
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.	
		Design and Procedures			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcome.	
Additional Comments:	This evaluat	ion was for the progeny assessment.			
Overall Qualit	ty Detern	nination	Medium		

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates on c	development, reproduc	tion, fat metabolism and lifespan in Daphnia magna. Science of the Total
Duration: Exposure Route,	Environmen Overall Dura Aquatic (free	t 654:969-977. ation: 0 - 4 days (0-96h); Exposure Duration: shwater); Water; Not determined by study autl	0 - 4 days (0-96h) hors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 5043468	Arthropods; <i>Daphnia magna</i> ; Embryo yl phthalate (DEHP)		
Domain	0010100	Metric	Rating	Comments
Domain 1: Test Substar	nce	mente	Truting	Commonits
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	High Low High	The chemical was identified by name and CAS#. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as >99%.
Domain 2. Test Design				
Domani 2. Test Design	Metric 4: Metric 5: Metric 6:	Negative Controls Negative Control Response Randomized Allocation	High Low Low	Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was not reported. Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	paracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure (96 hr) was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.
Domain 4: Test Organia	sm			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.

Diethylhexyl Phthalate

HERO ID: 5043468 Table: 3 of 5

			continued from previous pa	ge		
Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates o	n development, reproduction	, fat metabolism and lifespan in Daphnia magna. Science of the Total		
Duration:	Environment 654:969-977. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)					
Exposure Route, Modia Dath	Aquatic (free	snwater); water; Not determined by study a	iuthors (i.e., chemical of inte	est in exposure water, but unable to determine exact uptake route)		
Tava Species Age	Invertebrate	Invertebrate: Arthropode: Dephyia magna; Embryo				
Health Outcome	Mortality	Artinopous, Daprina magna, Emoryo				
Chemical:	Monancy Di-ethylbexyl phthalate (DEHP)					
HERO ID:	5043468					
Domain		Metric	Rating	Comments		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Uninformative	Data presentation was inadequate.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This was for	organisms with ephippia.				
Overall Quali	ty Detern	nination	Uninformative			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates o	n development, r	eproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total
Duration: Exposure Route,	Overall Dura Aquatic (free	ation: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study a	n: 0 - 4 days (0-9 authors (i.e., chen	6h) hical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Nutritional & Di-ethylhexy	Arthropods; <i>Daphnia magna</i> ; Juvenile & Metabolic vl phthalate (DEHP)		
HERO ID:	5043468	() - Printanie (2)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.
	Metric 2: Metric 3:	Test Substance Source Test Substance Purity	Low High	The test substance identity was not analytically verified by the performing laboratory. Chemical purity was reported as >99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.
		Repleates per Group		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.

Domain 6: Confounding / Variable Control

Diethylhexyl Phthalate

Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates of	n development, re	production, fat metabolism and lifespan in Daphnia magna. Science of the Total		
	Environmen	t 654:969-977.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-96	h)		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chemi	cal of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Nutritional & Metabolic					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5043468					
Domain		Metric	Rating	Comments		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This portion	of the evaluation was for lipid accumulatio	n.			
Overall Qualit	ty Detern	nination	Medium			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates on c	levelopment, reproduc	tion, fat metabolism and lifespan in Daphnia magna. Science of the Total				
Duration	Environmen Overall Dura	t 654:969-977. ation: 0 - 4 days (0-96h): Exposure Duration: 1	0 - 4 days (0-96h)					
Exposure Route.	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media. Path:								
Taxa, Species, Age:	Invertebrate:	Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Mortality	I I I I I I I I I I I I I I I I I I I						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5043468							
Domain		Metric	Rating	Comments				
Domain 1: Test Substa	nce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.				
Domain 2: Test Design	1							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups				
	intenite of		2011					
Domain 3: Exposure C	haracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured				
		Concentration	2011					
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 hr) was reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.				
		Spacing of Exposure Levels						
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.				
Domain 4: Test Organi	sm							
U	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15.	Conditions Number of Organisms and	Medium	The number of exposure groups and the spacing of exposure levels were suitable				
	Weute 15.	Replicates per Group	Wiedrum	The number of exposure groups and the spacing of exposure levels were suitable.				
		represed per Group						
Domain 5: Outcome A	ssessment							
	Metric 16:	Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.				
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-				
		Assessment		ited.				

Diethylhexyl Phthalate

HERO ID: 5043468 Table: 5 of 5

continued from previous page								
Study Citation:	Seyoum, A.,	Pradhan, A. (2019). Effect of phthalates o	n development, reproduction	, fat metabolism and lifespan in Daphnia magna. Science of the Total				
Duration: Exposure Route,	Environment Overall Dura Aquatic (free	Environment 654:969-977. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	5043468							
Domain		Metric	Rating	Comments				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.				
Domain 7: Data Present	tation and Anal	ysis						
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.				
	Metric 22:	Reporting of Data	Uninformative	Data presentation was inadequate.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None							
Overall Quali	ty Detern	nination	Uninformative					

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Daphnia magna</i>; Juvenile Immobilization Di-ethylhexyl phthalate (DEHP) 5498837 						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).			
	Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).			
	Metric 5:	Negative Control Response	High	No control mortality was observed in the control group treated with deionized water or acetone during the 48-h exposure.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered con- sistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and Daphnia magna juveniles were placed in a 100-mL glass beaker containing 100 mL of test solution.			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured and were similar to nominal concentrations, except for nominal concentration 0.06 mg/L for which the authors reported 0.00581 mg/L (given that the rest of the measured concentrations were very similar to the nominal concentrations, it is unclear if the reported value of 0.00581 mg/L was a typo with an extra 0). Moreover, there are no details of when the measured concentrations were taken, and it appears that they were not taken repeatedly such as at the beginning, throughout, and at the end of the study.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (acute toxicity in Daphnia magna).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Juveniles (< 24 h): The number of exposure groups and spacing of exposure levels were suitable for a dose response: 0.4, 0.6, 0.8, 1.0, and 1.2 mg/ L and a blank control and a solvent control (0.004% acetone, v/v).			
		Cont	tinued on nex	t page			

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Diethylhexyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path:	Study Citation:Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phtha activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental ContaminationDuration:Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)Exposure Route,Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, bu					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Immobilization Di-ethylhexyl phthalate (DEHP) 5498837					
Domain		Metric	Rating	Comments		
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in Daphnia magna.		
Domain 4. Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species (Daphnia magna) and age newly hatched juveniles (< 24 h)] for the study type. Moreover, the authors reported following test guidelines of the OECD Test No. 202: Daphnia sp. Acute Immobilisation Test.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to charac- terize toxicological effects. Five replicates were conducted for each treatment concen- tration (blank control, solvent control, plus DEHP concentrations). In each replicate, ten Daphnia magna juveniles were placed in a 100-mL glass beaker containing 100 mL of test solution, and a total of 50 organisms were exposed to each concentration in the DEHP gradient.		
Domain 5. Outcome As	accoment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	The authors reported following test guidelines of the OECD (2004), which is Test No. 202: Daphnia sp. Acute Immobilisation Test. In addition, the authors reported that the exposures were static, nonrenewal tests and that the test animals were not fed. However, the authors did not describe specific environmental conditions such as water quality control parameters taken during the test.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (organisms were considered immobile if they would not swim within 15 s after gentle agitation) was reported and adequate for the intended outcome of interest (immobilization to determine acute toxicity, LC50).		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported, and there was no evidence that it was not assessed consistently across study groups.		
Domain 6: Confounding	y / Variable Cor	atrol				
2 onium of Comounding	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There were no reported differences among the study groups in environmental conditions.		

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page				
Study Citation:	Wang, Y., W activity and	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in invenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Immobilizat	ion						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5498837							
Domain		Metric	Rating	Comments				
Domain 7: Data Present	ation and Anal Metric 21: Metric 22:	ysis Statistical Methods Reporting of Data	High Medium	Statistical methods were adequately described. The lethal concentrations such as LC50 and the 95% confidence limitswere calculated by Probit analysis using SPSS 21. The authors did not report the raw or mean immobilization results for each treatment group. They instead apported LC50 values at 24 and 48 hours, and they expected the				
				effect/correlation of DEHP concentration and exposure duration on acute toxicity.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments: The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipidperoxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in Daphnia magna newly hatched juveniles (< 24 h) and adults (> 96 h). This form was used to evaluate the acute toxicity (immobilization) in newly hatched juveniles (< 24 h).								
Overall Qualit	Overall Quality Determination High							

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Study Citation: Duration: Exposure Route, Media, Path:	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Di-ethylhexy 5498837	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5498837						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).				
	Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).				
	Metric 5:	Negative Control Response	High	The biological responses (oxidative stress and antioxidant gene expression) were re- ported and adequate for the control group.				
	Metric 6:	Randomized Allocation	Medium	Authors reported that for the subacute exposures to examine antioxidant activity and gene expression analyses, organisms were randomly assigned to experimental units (5 L beakers).				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered con- sistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and Daphnia magna adults were placed in a 5L glass beaker containing 5L of test solution.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure (48 h) was reported and suitable for the study type (antiox- idant activity and gene expression in Daphnia magna).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Adults (>96 h): The number of exposure groups and spacing of exposure levels were suitable for the antioxidant activity and gene expression assessment: 0.06 and 0.1 mg/L and a blank control and a solvent control (0.004% acetone, v/v).				
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in Daphnia magna.				

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Environmental Hazard Evaluation

HERO ID: 5498837 Table: 2 of 4

		cont	inued from p	revious page				
Study Citation: Duration: Exposure Route, Modia Pathy	 Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzym activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) 							
Taxa, Species, Age:	Invertebrate:	Arthropods: Daphnia magna: Adult						
Health Outcome: Chemical: HERO ID:	Mechanistic Di-ethylhexy 5498837	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5408927						
Domain		Metric	Rating	Comments				
Domain 4: Tast Organ	iam							
Domain 4. Test Organ	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species (Daphnia magna) and age (adults (> 96 h)] for the study type (subacute antioxidant effects of DEHP). Moreover, Daphnia magna is an adequate species used in toxicity tests and approved by OECD and EPA.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there was no evidence that they were not the same for control and exposed organisms.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to charac- terize subacute toxicological effects. 4 replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each repli- cate, a total of 500 adults were placed in 5-L glass beaker. At 24 and 48 h, 250 living organisms were collected from each beaker as a replicate and divided into two samples. 100 individuals were used to measure MDA content and enzyme activity, and 150 indi- viduals were used for RT-PCR analysis.				
Domain 5: Outcome A	ssessment							
	Metric 16:	Adequacy of Test Conditions	Low	The authors reported that for the acute toxicity studies they followed test guidelines of the OECD (2004), which is Test No. 202: Daphnia sp. Acute Immobilisation Test. However, the authors did not offer details on environmental conditions for the subacute experiments.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (lipid peroxidation levels, total antioxidant ca- pacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity, and catalase and glutathione-S-transferase mRNA levels) was reported and adequate for the intended outcome of interest (effects of DEHP on antioxidant responses).				
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported and there was no evidence that it was not assessed consistently across study groups.				
Domain 6: Confoundir	ng / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.				
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Diethylhexyl Phthalate

		contir	ued from p	previous page			
Study Citation:	Wang, Y., W	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gone expression in invanile and adult Daphnia mana. Archives of Environmental Contamination and Taxioology 75(1):145–156					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	S (0-96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:				•			
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Adult					
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cell sign	naling/funct	on			
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5498837						
Domain		Metric	Rating	Comments			
	Metric 21:	Statistical Methods	High	Data were presented as mean \pm standard deviation (SD). Student's t test was performed for comparisons using Excel. A p value < 0.05 was regarded as statistically significant, whereas p < 0.01 was considered as extremely significant.			
	Metric 22:	Reporting of Data	High	The authors reported mean \pm standard deviation (SD) for all treatments groups and all antioxidant responses assessed. One minor caveat was that there was no clarification on whether the control group reported in the results (text and figures) was the water control or the solvent control.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipid peroxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in Daphnia magna newly hatched juveniles (< 24 h) and adults (> 96 h). This form was used to evaluate oxidative stress and antioxidant gene expression in adults (> 96 h).						

Overall Quality Determination

High

Study Citation:	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme							
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	Invertebrate							
Health Outcome:	Mechanistic-	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function						
Chemical:	Di-ethylhexy	l phthalate (DEHP)	6					
HERO ID:	5498837							
Domain Domain 1: Test Substand	22	Metric	Rating	Comments				
Domain 1: Test Substand	Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).				
	Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).				
	Metric 5:	Negative Control Response	High	The biological responses (oxidative stress and antioxidant gene expression) were re- ported and adequate for the control group.				
	Metric 6:	Randomized Allocation	Medium	Authors reported that for the subacute exposures to examine antioxidant activity and gene expression analyses, organisms were randomly assigned to experimental units (5 L beakers).				
Domain 3: Exposure Ch	aracterization							
2 onium of 2nposine on	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and Daphnia magna juveniles were placed in a 5L glass beaker containing 5L of test solution.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (antioxidant activity and gene expression in Daphnia magna).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Juveniles (< 24 h): The number of exposure groups and the spacing of exposure levels were suitable for the antioxidant activity and gene expression assessment: 0.06 and 0.1 mg/L and a blank control and a solvent control (0.004% acetone, v/v).				
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in Daphnia magna.				

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Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 5498837 Table: 3 of 4

		cont	tinued from p	previous page			
Study Citation: Duration:	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Media. Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5498837						
Domain		Metric	Rating	Comments			
Domain 4: Test Organi	sm						
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species (Daphnia magna) and age (adults (< 24 h)] for the study type (subacute antioxidant effects of DEHP). Moreover, Daphnia magna is an adequate species used in toxicity tests and approved by OECD and EPA.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to charac- terize subacute toxicological effects. 4 replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each repli- cate, a total of 600 juveniles were placed in 5-L glass beaker. At 24 and 48 h, 250 living organisms were collected from each beaker as a replicate and divided into two samples. 100 individuals were used to measure MDA content and enzyme activity, and 150 indi- viduals were used for RT-PCR analysis.			
Domain 5: Outcome A	ssessment						
Domain 5. Outcome A	Metric 16:	Adequacy of Test Conditions	Low	The authors reported that for the acute toxicity studies they followed test guidelines of the OECD (2004), which is Test No. 202: Daphnia sp. Acute Immobilisation Test. However, the authors did not offer detail on environmental conditions for the subacute experiments.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (lipid peroxidation levels, total antioxidant ca- pacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity, and catalase and glutathione-S-transferase mRNA levels) was reported and adequate for the intended outcome of interest (effects of DEHP on antioxidant responses).			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported, and there is no evidence that it was not assessed consistently across study groups.			
Domain 6: Confoundir	ng / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no reported differences among the study groups in environmental conditions.			
Domain 7: Data Preser	ntation and Anal	vsis					
		<u>´</u> Con	tinued on new	xt nage			
		Con	macu on ne				

May 2025

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

HERO ID: 5498837 Table: 3 of 4

		contin	ued from p	previous page			
Study Citation:	Wang, Y., W activity and	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156.					
Duration:	Overall Dura	ition: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	s (0-96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Cell sign	aling/funct	ion			
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5498837						
Domain		Metric	Rating	Comments			
	Metric 21:	Statistical Methods	High	Data were presented as mean \pm standard deviation (SD). Student's t test was performed for comparisons using Excel. A p value < 0.05 was regarded as statistically significant, whereas p < 0.01 was considered as extremely significant.			
	Metric 22:	Reporting of Data	High	The authors reported mean \pm standard deviation (SD) for all treatments groups and all antioxidant responses assessed. One minor caveat was that there was no clarification on whether the control group reported in the results (text and figures) was the water control or the solvent control.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipid peroxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in Daphnia magna newly hatched juveniles (< 24 h) and adults (> 96 h). This form was used to evaluate oxidative stress and antioxidant gene expression in newly hatched juveniles (< 24 h).						

Overall Quality Determination

High

Study Citation:	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme							
Duration	activity and	activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156.						
Duration: Exposure Route.	Aquatic (free	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media, Path:	riquite (iie)	situation, water, not determined by study	uutions (1.e.,	enclinear of interest in exposure water, but anable to determine exact aptake route)				
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Adult						
Health Outcome:	Immobilizati	Immobilization						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	5498837							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce	Trat Cubatana Islandita	II: -l-					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81- 7).				
	Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".				
Domain 2: Test Design								
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).				
	Metric 5:	Negative Control Response	High	No control mortality was observed in the control group treated with deionized water or acetone during the 48-h exposure.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 2. Eurocum Ch	anastanization							
Domain 5. Exposure Ch	Metric 7	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare				
	metric /.	Preparation	Low	test concentrations.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered con- sistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and Daphnia magna adults were placed in a 100- mL aloss basics containing 100 mL of test colution				
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured and were similar to nominal concentrations, except for nominal concentration 0.06 mg/L for which the authors reported 0.00581 mg/L (given that the rest of the measured concentrations were very similar to the nominal concentrations, it is unclear if the reported value of 0.00581 mg/L was a typo with an extra 0). Moreover, there are no details of when the measured concentrations were taken, and it appears that they were not taken repeatedly such as at the beginning, throughout, and at the end of the study.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (acute toxicity in Daphnia magna).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Adults (>96 h): The number of exposure groups and spacing of exposure levels were suitable for a dose response: 0.2, 0.3, 0.4, 0.5, and 0.6 mg/L and a blank control and a solvent control (0.004% acetone, v/v).				
		Con	tinued on nex	xt page				
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Diethylhexyl Phthalate

Study Citation:	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, O., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme					
Duration: Exposure Route, Media. Path:	 activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake reference of the study authors (i.e., ch					
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Adult				
Health Outcome:	Immobilizati	ion				
Chemical: HERO ID:	Di-ethylhexy 5498837	l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L . In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in Daphnia magna.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species (Daphnia magna) and age (adults (> 96 h)] for the study type. Moreover, the authors reported following test guidelines of the OECD Test No. 202: Daphnia sp. Acute Immobilisation Test.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects. Five replicates were conducted for each treatment concentra- tion (blank control, solvent control, plus DEHP concentrations). In each replicate, ten Daphnia magna adults were placed in a 100-mL glass beaker containing 100 mL of test solution, and a total of 50 organisms were exposed to each concentration in the DEHP gradient.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	The authors reported following test guidelines of the OECD (2004), which is Test No. 202: Daphnia sp. Acute Immobilisation Test. In addition, the authors reported that the exposures were static, nonrenewal tests and that the test animals were not fed. However, the authors did not describe specific environmental conditions such as water quality control parameters taken during the test.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (organisms were considered immobile if they would not swim within 15 s after gentle agitation) was reported and adequate for the intended outcome of interest (immobilization to determine acute toxicity, LC50).		
	Metric 18:	Consistency of Outcome Assessment	Medium	The outcome assessment protocol was reported, and there is no evidence that it was not assessed consistently across study groups.		
Domain 6: Confounding	y / Variable Cor	ntrol				
2 onian of Comounding	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There were no reported differences among the study groups in environmental conditions.		

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		contin	nued from p	revious page			
Study Citation:	Wang, Y., W activity and	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult Daphnia magna. Archives of Environmental Contamination and Toxicology 75(1):145-156.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	hwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Adult					
Health Outcome:	Immobilizati	on					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5498837						
Domain		Metric	Rating	Comments			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described. The lethal concentrations such as LC50 and the 95% confidence limitswere calculated by Probit analysis using SPSS 21.			
	Metric 22:	Reporting of Data	Medium	The authors did not report the raw or mean immobilization results for each treatment group. They instead reported LC50 values at 24 and 48 hours, and they reported the effect/correlation of DEHP concentration and exposure duration on acute toxicity.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments: The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipidperoxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in Daphnia magna newly hatched juveniles (< 24 h) and adults (> 96 h).							
Overall Qualit	y Detern	nination	High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.							
Duration: Exposure Route, Media. Path:	Environment Overall Dura Aquatic (free	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile						
Health Outcome:	ADME (biot	ADME (biotransformation)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
	1334040							
Domain Domain 1: Test Substan	<u></u>	Metric	Kating	Comments				
Domain 1. Test Substan	Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
U	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	naracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure Administration	Low	Few details of the exposure administration were reported, and exposures were adminis- tered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	Low	Only one treatment was reported.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organis	m							
C C	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.				
Domain 5: Outcome As	sessment	· ·						
20main 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.				
		(Continued on next page	·				

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Diethylhexyl Phthalate

HERO ID: 1334646 Table: 1 of 1

		0	ontinued from previous	page				
Study Citation:	Jr, Mayer, F	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.						
	Environment	Environmental Research 6(1):84-90.						
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10) days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile							
Health Outcome:	ADME (biot	ransformation)						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	1334646							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for outcome assessment were				
		Assessment		provided.				
Domain 6: Confounding	/ Variable Co	ntrol						
2 children of Contourioung	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmenta conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.				
Domain 7: Data Presenta	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was not performed.				
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for the sampling period.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None							

Study Citation: Duration: Exposure Route,	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	-			-	
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	679904				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The DEHP was identified by nomenclature (Table 1a).	
	Metric 2:	Test Substance Source	High	The sources were identified (Table 1a).	
	Metric 3:	Test Substance Purity	High	"These samples are commercial products with stated purities in excess of 99.5 (w/w)."	
Domain 2. Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	"Ten replicate beakers each containing 1 Daphnia were used for the control, dispersant control (10 mg/l), and for each phthalate treatment."	
	Metric 5:	Negative Control Response	High	No effects were observed in any controls.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3 [.] Exposure Ch	aracterization				
2 011411 01 2.1905410 01	Metric 7:	Experimental System/Test Media Preparation	High	A large amount of surfactant (10x the concentration of phthalate) was used to ensure that the phthalate was suspended in the test solution.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The authors report that for the chronic tests, the actual recovered concentrations resembled nominal when tested in freshly prepared treatment solution and in solution held for the duration of the experiment (Tables 6 & 7).	
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency were appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This is a limit test ("3 orders of magnitude" above solubility limit, no adverse effects noted in any exposure). Though DEHP was studied at two concentrations (0.25 mg/L and 1 mg/L) in chronic tests, both concentrations are far above the solubility limit.	
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations exceeded the solubility limit but were within the dispersibility limit with the dispersants used, as noted by the authors.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	"The test organism was the freshwater crustacean Daphnia magna Strauss, derived from continuous laboratory cultures."	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	"The cultures were maintained under similar conditions (water, temperature, photope- riod, and feeding) to those described for the reproduction test."	
	Metric 15:	Number of Organisms and Replicates per Group	N/A	This is a limit test.	

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Brown, D., C	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia manage. Chemosphere 36(6):1367, 1379					
Duration:	Overall Dura	ation: 11 - 21 days: Exposure Duration: 11	- 21 days	le 50(0).1507-1577.			
Exposure Route.	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	1						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	679904						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. "The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room, and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."			
	Metric 17:	Outcome Assessment Methodology	High	Mortality was evaluated as immobilization.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

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Study Citation:	Brown, D., C	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Di-ethylhexy 679904	Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic l phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The DEHP was identified by nomenclature (Table 1a).			
	Metric 2:	Test Substance Source	High	The sources were identified (Table 1a).			
	Metric 3:	Test Substance Purity	Hıgh	"These samples are commercial products with stated purities in excess of 99.5 (w/w)."			
Domain 2: Test Design							
2 oniani 21 1000 2 001gn	Metric 4:	Negative Controls	High	"Ten replicate beakers each containing 1 Daphnia were used for the control, dispersant control (10 mg/l), and for each phthalate treatment."			
	Metric 5:	Negative Control Response	High	No effects were observed in any controls.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
	, . . .						
Domain 3: Exposure Cha	aracterization	E/T/M-di-	TT: _1.				
	Metric 7:	Propagation	пign	A large amount of surfactant (10x the concentration of phinalate) was used to ensure that the phthalate was suspended in the test solution			
	Metric 8.	Consistency of Exposure	High	Exposures were administered consistently across study groups			
	metrie 6.	Administration	mgn	Exposures were administered consistently deross study groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	The authors report that for the chronic tests, the actual recovered concentrations resem- bled nominal when tested in freshly prepared treatment solution and in solution held for the duration of the experiment (Tables 6 & 7).			
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency were appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This is a limit test ("3 orders of magnitude" above solubility limit, no adverse effects noted in any exposure). Though DEHP was studied at two concentrations (0.25 mg/L and 1 mg/L) in chronic tests, both concentrations are far above the solubility limit.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations exceeded the solubility limit but were within the dispersibility limit with the dispersants used, as noted by the authors.			
Domain 4: Test Organise	m						
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	"The test organism was the freshwater crustacean Daphnia magna Strauss, derived from continuous laboratory cultures."			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	"The cultures were maintained under similar conditions (water, temperature, photope- riod, and feeding) to those described for the reproduction test."			
	Metric 15:	Number of Organisms and Replicates per Group	N/A	This was a limit test.			

Domain 5: Outcome Assessment

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Diethylhexyl Phthalate

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Study Citation:	Brown, D., C dispersions of	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.						
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile							
Health Outcome:	Reproductive	e/Teratogenic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	679904							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ""The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room, and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."				
	Metric 17:	Outcome Assessment Methodology	High	"From day 5, observations were also made daily for the presence of offspring (termed F1 generation)."				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	y / Variable Cou	ntrol						
Domain of Companying	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	veis						
Domain 7. Dua 110501	Metric 21.	Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None							
Overall Quali	ty Detern	nination	High					

Study Citation:	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379						
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Developmen Di-ethylhexy	Arthropods; <i>Daphnia magna</i> ; Juvenile t/Growth /l phthalate (DEHP)					
HERO ID:	679904	Metric	Pating	Comments			
Domain 1: Test Substan	re .	Metric	Katilig	Comments			
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	The DEHP was identified by nomenclature (Table 1a)			
	Metric 2:	Test Substance Source	High	The sources were identified (Table 1a)			
	Metric 3:	Test Substance Purity	High	"These samples are commercial products with stated purities in excess of 99.5 (w/w)."			
Domain 2: Test Design	Metric 4:	Negative Controls	High	"Ten replicate beakers each containing 1 Daphnia were used for the control, dispersant control (10 mg/l), and for each phthalate treatment."			
	Metric 5:	Negative Control Response	High	No effects were observed in any controls.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2. Euroques Ch	anastanization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	A large amount of surfactant (10x the concentration of phthalate) was used to ensure that the phthalate was suspended in the test solution.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The authors report that for the chronic tests the actual recovered concentrations resembled nominal when tested in freshly prepared treatment solution and in solution held for the duration of the experiment (Tables 6 & 7)			
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency were appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This is a limit test ('3 orders of magnitude" above solubility limit, no adverse effects noted in any exposure). Though DEHP was studied at two concentrations (0.25 mg/L and 1 mg/L) in chronic tests, both concentrations are far above the solubility limit.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations exceeded the solubility limit but were within the dispersibility limit with the dispersants used, as noted by the authors.			
Domain 4: Test Organis	m						
2 chian ii rest organisi	Metric 13:	Test Organism Characteristics	High	"The test organism was the freshwater crustacean Daphnia magna Strauss, derived from continuous laboratory cultures."			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	"The cultures were maintained under similar conditions (water, temperature, photope- riod and feeding) to those described for the reproduction test"			
	Metric 15:	Number of Organisms and Replicates per Group	N/A	This is a limit test.			

Domain 5: Outcome Assessment

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page				
Study Citation:	Brown, D., C dispersions of	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the Daphnia magna. Chemosphere 36(6):1367-1379.						
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (1.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	679904							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ""The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."				
	Metric 17:	Outcome Assessment Methodology	High	"At the end of the test (21 days), the length of each surviving Po generation Daphnia was measured (apex of helmet to base of spine) using a microscope with a calibrated graticule."				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	r / Variable Cou	atrol						
Domain of Comoundanty	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None	T to the transformed second	<u> </u>	¥ toto restrict to				

Overall Quality Determination

High

Study Citation:	Brown, D.,	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on					
Duration: Exposure Route, Media, Path:	the reproduc Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1334281						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
c c	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	araatarization						
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media	Madium	The experimental system and/or test modio properation methods were adequately re-			
	Metric 7.	Preparation	Wiedium	ported.			
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered			
		Administration	U	consistently across study groups.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
C C	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects, but no effect was reported.			
Domain 5. Outrans							
Domain 5: Outcome As	Sessment	A dequeey of Test Conditions	IIIah	Our sign and the distance of the interview of the second s			
	Matrie 17	Adequacy of Test Conditions	High Madiation	Organism environmental conditions were conducive to the maintenance of health.			
	Metric 1/:	Outcome Assessment Methodology	Medium	I ne outcome assessment methodology partially addressed the outcome of interest.			
		Conti	nued on next pa	ge			

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426. **Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1334281 Domain Metric Rating Comments Metric 18: Consistency of Outcome Medium Details regarding the execution of the study protocol for outcome assessment were limited. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Though statistical analysis was not conducted, there was no effect seen at any concentration. Metric 22: Continuous data were presented without measures of variability. Reporting of Data Low Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes. Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on							
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile						
Health Outcome:	ADME (biot	ADME (biotransformation)						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	1334281							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		TT' 1					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
	Metric 2: Metric 3:	Test Substance Durity	Low	The source was not reported. The purity and/or grade of the test substance were not reported				
	Wietric 5.	Test Substance Furity	LOw	The purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-				
	Metric 8.	Consistency of Exposure	High	Details of exposure administration were reported and exposures were administered				
		Administration		consistently across study groups.				
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type				
	Metric 11:	Number of Exposure Groups/	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
			U					
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects, but no effect was reported.				
Domain 5: Outcome Ass	sessment	• •						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.				
		Conti	nued on next pa	ge				

		contin	ued from previo	us page		
Study Citation:	Brown, D., 7 the reproduc	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on he reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426.				
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	thors (i.e., chem	cal of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1334281					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	/ Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Presenta	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Though statistical analysis was not conducted, there was no effect seen at any concentra- tion.		
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	y Detern	nination	Medium			

Study Citation:	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on					
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	1334281					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
6	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric /:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-		
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered		
		Administration	ing.	consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
				· · ·		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects, but no effect was reported.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
	Continued on next page					

	continued from previous page					
Study Citation:	Brown, D., ' the reproduc	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentration. Chemosphere 11(4):417-426.				
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1334281					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High Medium	There were no reported differences among the study groups in environmental conditions. There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quali	Overall Quality Determination Medium					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jr, Mayer, F	E., Sanders, H. O., Walsh, D. F. (1973).	Toxicity, residue	dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.
Duration: Exposure Route, Media. Path:	Environment Overall Dura Aquatic (free	tal Research 6(1):84-90. ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study	- 21 days authors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Reproductive	e/Teratogenic		
Chemical:	Di-ethylhexy	vl phthalate (DEHP)		
HERO ID:	1334646			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identify was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
6	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	High	The biological response (reproduction) of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of exposure administration were reported, and exposures were adminis- tered consistently across study groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a
		Spacing of Exposure Levels		dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Low	The number of test replicates was not reported.
		Replicates per Group		
Domain 5: Outcome Ass	sessment			
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade-
	Metric 17:	Outcome Assessment Methodology	Low	quate. The outcome assessment methodology was not clearly reported.
		Con	tinued on next pa	ge

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HERO ID: 1334646 Table: 1 of 1

		contir	nued from previ	bus page		
Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973). T	Toxicity, residue	dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.		
	Environmen	Environmental Research 6(1):84-90.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	- 21 days			
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1334646					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not		
		Assessment		reported.		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Deterr	nination	Medium			

Study Citation:	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction				
Duration: Exposure Route, Media, Path:	of daphnia-n Overall Dura Aquatic (free	nagna. Environmental Toxicology and Che ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study	- 21 days authors (i.e.,	01-208. chemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	1334951				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.	
	Metric 2:	Test Substance Source	High	The test substance batch number and identity were analytically verified by the perform- ing laboratory.	
	Metric 3:	Test Substance Purity	High	The purity was reported as 97%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Cha	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate for a dose response.	
	Metric 12:	Testing at or Below Solubility Limit	Medium	The high concentration (811 ug/L) was over the solubility limits (270 ug/L) listed in the final scope for DEHP.	
Domain 4: Test Organisr	n				
c	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but the source was not reported.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and replicates were reported and sufficient to characterize	
		Replicates per Group		toxicological effects.	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
		Cont	inued on nex	t page	

		contir	nued from p	previous page		
Study Citation:	Knowles, C.	O., Mckee, M. J., Palawski, D. U. (1987).	Chronic effe	ects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction		
	of daphnia-n	nagna. Environmental Toxicology and Cher	mistry 6(3):2	201-208.		
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days			
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1334951					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures	0			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7. Data Dragant	otion and Anal	visio				
Domain 7. Data Fresent	Motrio 21:	ysis Statistical Mathada	Uich	Statistical matheda wave adagestaly described		
	Metric 21.	Perperting of Data	High Ligh	Statistical methods were adequately described.		
	Metric 22.	Events of Linear entrol Outcomes	High LU-h	Data for exposure-related midings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	i nere were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	High			

Study Citation: Duration: Exposure Route,	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Di-ethylhexy 1334951	Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic el phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.	
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Hıgh	The purity was reported as 97%.	
Domain 2: Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12:	Testing at or Below Solubility Limit	Medium	The high concentration (811 ug/L) was over the solubility limits (270 ug/L) listed in the final scope for DEHP.	
Domain 4: Test Organis	m				
2 sinum 1. rest organis	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but the source was not reported.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and replicates were reported and sufficient to characterize	
		Replicates per Group		toxicological effects.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.	
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Continued on next page					

	continued from previous page					
Study Citation:	Knowles, C.	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction				
	of daphnia-n	of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.				
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1334951					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures	TT' 1			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	incure 25.		mgn			
Additional Comments:	None					
Overall Quali	Overall Quality Determination High					

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HERO ID: 1334951 Table: 3 of 4

Study Citation:	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphaia magna. Environmental Toxicology and Chemistry 6(3):201–208					
Duration: Exposure Route, Media Path:	Overall Dur Aquatic (fre	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome:	Invertebrate Mechanistic	Arthropods; <i>Daphnia magna</i> ; Juvenile Biomarkers (exposure and effect)				
HERO ID:	1334951	yi pitilalate (DEHF)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	The purity was reported as 97%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain D. Exposure on	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The high concentration (811 ug/L) was over the solubility limit (270 ug/L) listed in the final scope for DEHP.		
Domain 4. Test Organis	n					
Domain 4. Test Organisi	Metric 13.	Test Organism Characteristics	Medium	The test organisms were adequately described, but the source was not reported		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and replicates were reported and sufficient to characterize		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.		
		Cont	inued on nex	t page		

continued from previous page						
Study Citation:	Knowles, C.	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction				
	of daphnia-n	of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.				
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1334951					
Domain		Metric	Rating	Comments		
		4.1				
Domain 6: Confounding	g / Variable Col		TT' 1			
	Metric 19:	Confounding variables in Test	High	There were no reported differences among the study groups.		
	Matria 20.	Design and Procedures	Uich	These wars no differences among around that could influence the outcome accessment		
	Metric 20.	Outcomes Offenated to Exposure	nigii	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This is an as	sessment for the biochemical component ar	nalysis.			
Overall Quali	Overall Quality Determination High					

Study Citation: Duration: Exposure Route,	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Behavioral Di-ethylhexy 1334951	Arthropods; <i>Daphnia magna</i> ; Juvenile /l phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.	
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	The purity was reported as 97%.	
Domain 2: Test Design					
2 oniuni 21 Test 2 esign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization		*** 1		
	Metric 7:	Experimental System/Test Media Preparation	Hıgh	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12:	Testing at or Below Solubility Limit	Medium	The high concentration (811 ug/L) was over the solubility limit (270 ug/L) listed in the final scope for DEHP.	
Domain 4: Test Organisi	n				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but the source was not reported.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and replicates were reported and sufficient to characterize	
		Replicates per Group			
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.	
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Continued on next page					

	continued from previous page						
Study Citation:	Knowles, C.	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction					
	of daphnia-n	of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days				
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1334951						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.			
		Design and Procedures	TT' 1				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7. Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
		• •	U	•			
Additional Comments:	This form is	for surfacing behavior.					
Overall Quality Determination High							

Study Citation: Duration:	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days							
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	680120							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
	Metric 2:	Test Substance Source	Low	The test substance identity was NOT analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	The purity was reported as >95%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Damain 2. European Ch								
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Madia	Madium					
	Metric 7:	Preparation	Medium	in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered				
	Matria O.	Administration Measurement of Test Substance	High	consistently across study groups.				
	Metric 9.	Concentration	High	exposure concentrations were measured using appropriate analytical technologies and methods				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organis	m Matria 12.	Trat Organism Changeteristics	II: -1-					
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.				
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.				
		Cont	tinued on nex	t page				

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HERO ID: 680120 Table: 1 of 2

		conti	nued from p	orevious page			
Study Citation:	Rhodes, J. E rainbow trou	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	\mathbf{I}						
Taxa, Species, Age:	Invertebrate	; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	680120						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
	Assessment						
Domain 6: Confounding	y / Variable Co	ntrol					
Domain o. Comountaing	Metric 19	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	incure 19.	Design and Procedures	mgn	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7. Data Presant	totion and Anal	unin .					
Domain 7. Data Fresen	Motrio 21:	Statistical Matheda	Low	Statistical analysis was notformed but not described adaptately			
	Metric 21:	Statistical Methods	LOW	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	and were adequate.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quali	ty Deterr	nination	High				

Study Citation:	Rhodes, J. E rainbow trou	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976.					
Duration: Exposure Route, Media Path	Overall Dura Aquatic (free	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Di-ethylhexy 680120	Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic l phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was NOT analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The purity was reported as >95%.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	Medium	The experimental system and methods for preparation of the test media were described			
	Medile /.	Preparation	mean	in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies and methods			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
	^						
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
		T the second sec					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
		Cont	inued on nex	t page			

Diethylhexyl Phthalate

		contin	ued from p	previous page			
Study Citation:	Rhodes, J. E	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and					
Duration:	Overall Dur	Taillow trout (Oncomynenus mykrss). Environmental toxicology and Chemistry 14(11):1907-1970. Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days					
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media. Path:	i iquaio (iio	, in aller, i not accertained by stady a	unions (nei,				
Taxa. Species. Age:	Invertebrate:	Arthropods: Daphnia magna: Juvenile					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	/l phthalate (DEHP)					
HERO ID:	680120	680120					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quality Determination High							

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Study Citation:	Seyoum, A.,	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total						
Duration: Exposure Route, Media, Path:	Environment Overall Dura Aquatic (free	Invironment 654:969-977. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Invertebrate: Arthropods: Daphnia magna: Juvenile						
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	5043468							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization		-					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	Low	Only one exposure group was reported.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.				
Domain 4: Test Organia								
Domain 4: Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms				
	Wieure 14.	Conditions	Ingh	An pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Number of Organisms and	Medium	The number of organisms and replicates were suitable.				
		Replicates per Group						
Domain 5: Outcome Ag	sessment							
Domain 5. Outcome As	Metric 16.	A dequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported				
	Metric 17	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome				
	meure 17.	Sateome Assessment Methodology	ingn	The outcome assessment methodology reported the intended outcome.				
Continued on next page								

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Diethylhexyl Phthalate

HERO ID: 5043468 Table: 1 of 1

		contin	ued from previo	us page			
Study Citation:	Seyoum, A.,	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	uthors (i.e., chem	ical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5043468						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited			
Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups.							
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcome.			
Additional Comments:	This evaluat	on was for Daphnid length.					
Overall Quality Determination M			Medium				

Study Citation: Duration:	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path: Taxa, Species, Age: Health Outcome:	Invertebrate; Mortality	Arthropods; Daphnia magna; Adult				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1316195					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name (DEHP), but no further information was provided.		
	Metric 2:	Test Substance Source	High	The test substance was from General Electric Company, Hudson Falls, New York.		
	Metric 3:	Test Substance Purity	Low	The purity was not provided.		
Domain 2: Test Design						
-	Metric 4:	Negative Controls	High	A negative control was used (not a solvent control).		
	Metric 5:	Negative Control Response	Medium	Authors reported for DEHP that animals in the controls appeared to be trapped at the surface. While the authors claimed that this did not affect the survival of the controls, as evidenced by the $<10\%$ mortality in the controls.		
	Metric 6:	Randomized Allocation	Medium	Organisms were allocated in an unbiased manner.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Medium	The experimental system was a flow-through system with an "enhanced mixing process"		
		Preparation		to add in the chemical, which is poorly soluble in water. A solvent was not used.		
	Metric 8:	Consistency of Exposure	High	The exposure was administered consistently across groups.		
	Metric 9:	Administration Measurement of Test Substance	High	Test concentrations were measured weekly.		
	Metric 10.	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate - 21 days		
	Metric 11:	Number of Exposure Groups/	High	5 concentrations were tested.		
		Spacing of Exposure Levels	8			
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some concentrations exceeded the water solubility limit. An enhanced mixing technique was used to deliver the chemical in the flow-through system.		
Domain 4: Test Organis						
c	Metric 13:	Test Organism Characteristics	High	The source of the Daphnia was Springborn Bionomics.		
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimatization was not reported.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There is no description of how many replicates there were per treatment or how many animals there were per replicate. An old protocol is cited: "Protocol for conducting chronic toxicity tests with the water flea Daphnia magna)" developed at EG&G Bionomics {1982}.		

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Mortality Di-ethylhexyl phthalate (DEHP) 1316195						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	Metric 16: Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome	High High High	The environmental conditions were recorded and were consistent. Mortality was assessed weekly, and reproduction was assessed as the cumulative number of offspring. Outcomes were assessed consistently across groups.			
Domain 6: Confounding	/ Variable Cor	assessment					
	Metric 19:	Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Medium	Authors state that daphnia in the control and 0.29 mg/L exposure group were observed to be entrapped at the surface, which likely affected survival.			
	Wette 20.	Sucones enclated to Exposure	Low	to be entrapped at the surface, which likely affected survival.			
Domain 7: Data Presenta	ation and Anal Metric 21:	ysis Statistical Methods	High	Statistical tests were performed (not well described, authors cite an old method), and all data are presented in the tables for treatments and controls.			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High High	All data are presented in the tables for treatments and controls. Outcomes were satisfactorily described.			
Additional Comments:	None						
Overall Qualit	Overall Quality Determination High						

Study Citation: Duration: Exposure Route, Media Path:	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Di-ethylhexy 1316195	Arthropods; <i>Daphnia magna</i> ; Adult e/Teratogenic /l phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Low	The test substance was identified by name (DEHP), but no other information was pro- vided.	
	Metric 2: Metric 3:	Test Substance Source Test Substance Purity	High Low	The test substance was from General Electric Company, Hudson Falls, New York. The purity was not provided.	
Domain 2: Test Design			TT. 1		
	Metric 4: Metric 5:	Negative Controls Negative Control Response	High Medium	A negative control was used (not a solvent control). Authors reported for DEHP that animals in the controls appeared to be trapped at the surface, but overall survival of controls was acceptable.	
	Metric 6:	Randomized Allocation	Medium	Organisms were allocated in an unbiased manner.	
Domain 3: Exposure Ch	aracterization		M P		
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was a flow-through system with an "enhanced mixing process" to add in the chemical, which is poorly soluble in water. A solvent was not used.	
	Metric 8:	Administration Measurement of Test Substance	High	Test concentrations were measured weekly.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate - 21 days.	
	Metric 11:	Spacing of Exposure Groups/ Spacing of Exposure Levels	High	5 concentrations were tested. There were no effects on reproduction observed at the highest concentration (at the solubility limit).	
	Wietrie 12.	Testing at of Below Solubility Emilt	Wiedrum	was used to deliver the chemical in the flow-through system.	
Domain 4: Test Organisi	m				
	Metric 13:	Test Organism Characteristics	High	The source of the Daphnia was Springborn Bionomics.	
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimatization was not reported.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	There is no description of how many replicates there were per treatment or how many animals there were per replicate. An old protocol is cited: "Protocol for conducting chronic toxicity tests with the water flea Daphnia magna)" developed at EG&G Bionomics {1982}.	
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were recorded and were consistent.	
	Metric 17:	Outcome Assessment Methodology	High	Reproduction was assessed as the cumulative number of offspring.	
Continued on next page					

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1316195 Table: 2 of 2

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Bionomics,, Overall Dura Aquatic (free Invertebrate; Reproductivy Di-ethylhexy	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95. Overall Duration: 11 - 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Reproductive/Teratogenic					
HERO ID:	1316195						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Authors state that daphnia in the control and 0.29 mg/L exposure groups were observed to be entrapped at the surface.			
	Metric 20:	Outcomes Unrelated to Exposure	Low	Authors state that daphnia in the control and 0.29 mg/L exposure groups were observed to be entrapped at the surface, which likely affected survival.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical tests were performed (not well described, authors cite an old method), and all data are presented in the tables for the treatments and controls.			
	Metric 22:	Reporting of Data	High	All data are presented in the tables for the treatments and controls.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily described.			
Additional Comments:	None						
Overall Quality Determination			High				
PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.						
D (1	Environmen	Environmental Pollution $27(4):263-274$.					
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: > 21 d	lays				
Exposure Route, Modia Dath	Aquatic (fre	snwater); water; Not determined by study au	thors (i.e., chemical of in	iterest in exposure water, but unable to determine exact uptake route)			
Media, Path:	Invantahuata	Warma (a.a. Annalida Namatadaa), Daudu	oooluu lastauu Not A	anliaghla (a. a. fungi an alaga studiog) an Nat Danastad			
Taxa, Species, Age:	ADME (bio	; worms (e.g., Annends, Nenhalodes); Denard	ocoeium iacieum, Noi Aj	ppincable (e.g., fungi of algae studies) of Not Reported			
Chamical:	Di ethylbey	Di-ethylhexyl phthalate (DEHP)					
HFRO ID.	50542	yi pittialate (DETII)					
Domoin	57542	Matria	Datina	Commente			
Domain Domain 1. Test Substan		Менис	Kating	Comments			
Domain 1: Test Substan	Metric 1.	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylbexylphthalate (DEHP) No			
	Weute I.	Test Substance Identity	Weddulli	CASRN or structure were provided.			
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.			
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.			
Domain 2: Test Design	Matria 1.	Negative Controls	Uninformative	No pagative control group was reported			
	Metric 4.	Negative Control Response	N/A	No negative control group was reported.			
	Metric 5.	Regarive Control Response Pandomized Allocation	IN/A Low	No negative control group was reported.			
	Metric 0.	Kandolilized Allocation	LOW	No fandom anocation was reported.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media	Medium	Biomass loading and the placement of organisms in 20L tanks were not reported.			
		Preparation					
	Metric 8:	Consistency of Exposure	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP.			
		Administration		DEHP was dissolved in acetone prior to adding it to the system.			
	Metric 9:	Measurement of Test Substance	Hıgh	The test substance was measured by TLC and liquid scintillation. Final concentrations			
		Concentration		of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass balances were calculated. The mass			
				balance was DEHP + metabolites (phthalic acid and phthalic anhydride).			
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.			
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximately sol-			
				ubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.			
Domain 4: Test Organia	m						
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	Organisms were collected in the field. Age and say were not provided in the study			
	Metric 14	Acclimatization and Pretreatment	LOW	The study did not report acclimation or pretreatment			
	wiente 14.	Conditions	LUW	The study did not report accumation of pretreatment.			
	Metric 15:	Number of Organisms and	Low	No replicates were reported.			
		Replicates per Group		-			

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

continued from previous page							
Study Citation:	Sodergren, A Environment	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.					
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21 d	lays				
Exposure Route,	Aquatic (fres	hwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Dendrocoelum lacteum; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biotransformation)						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	59542						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.			
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment was described, but details of organism preparation for extraction was not described (euthanasia, if applicable, or harvesting of plant material).			
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.			
Domain 6: Confounding	/ Variable Con	itrol					
Domain of Comounding	Metric 19:	Confounding Variables in Test	Low	Multiple organisms were loaded into the same experimental tank which could affect 14C			
		Design and Procedures		DEHP uptake by each organism.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal at- trition or health outcomes unrelated to the exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Presenta	ation and Analy	ysis					
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.			
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.			
Additional Comments:	This study comesocosm. C	ollected 13 organisms (fish, invertebrates, One tank containing the organisms was comp	and plants) from unpollubleted. Fish were separate	uted streams, as well as the collection of water and sediment for the d in the tank. Toxicity was addressed as "the results indicate that DEHP			

may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites. The metabolites were phthalic acid and phthalic anhydride.

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Jr, Mayer, F Environment Overall Dura Aquatic (free	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	nvertebrate; Arthropods; Gammarus pseudolimnaeus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Chemical:	Di-ethylhexy	Viortality Di-ethylbexyl phthalate (DEHP)					
HERO ID:	1334646						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.			
	Metric 6:	Randomized Allocation	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	Concentrations of the test substance were not measured during the study.			
		Preparation		,			
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of the exposure administration were reported, and exposures were adminis- tered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and the spacing of expo- sure levels.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.			
Domain 4. Test Organic	m						
Domain +, Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of the test animals was not reported			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
		Conditions	2011				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.			

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

HERO ID: 1334646 Table: 1 of 1

			continued from previous pa	ge		
Study Citation:	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chemical of inter	rest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Gammarus pseudolimnaeus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1334646					
Domain	ain Metric Rating Comment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Uninformative			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.						
·	Environmen	tal Research 6(1):84-90.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days				
Exposure Route, Modia Dath:	Aquatic (free	quare (neshwater); water, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake foute)					
Tava Species Age	Invertebrate	nvertebrate: Arthropods: Gammarus pseudolimnaeus: Not Applicable (e.g. fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ADME (biotransformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1334646						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
	Wieule 7.	Preparation	Low	test concentrations.			
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported, and exposures were adminis-			
		Administration		tered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	Low	Only one treatment was reported.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	36 . 1 17	Conditions	T				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.			
		(Continued on next page .				

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Diethylhexyl Phthalate

HERO ID: 1334646 Table: 1 of 1

		сог	ntinued from previous	page		
Study Citation:	Jr, Mayer, F	r, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.				
D (1	Environment	Environmental Research 6(1):84-90.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 - 2	1 days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study auth	nors (i.e., chemical of i	nterest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Gammarus pseudolimnaeus; No	t Applicable (e.g., fung	gi or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1334646					
Domain	Metric Rating Comments					
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.		
Domain 6: Confounding	g / Variable Cor Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Low Medium	The study did not provide enough information to allow a comparison of environmental conditions. There was no information in the study to suggest differences among groups in animal		
Domain 7: Data Present	tation and Anal Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	Low Medium High	Statistical analysis was not performed. Data for exposure-related findings were presented for the sampling period.		
	Methe 25.	Explanation of Onexpected Outcomes	Ingn			
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Uninformativ	e		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Oil,, Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp Gammarus pulex (L) to					
Duration: Exposure Route, Media, Path:	toxicants wi Overall Dura Aquatic (frea	th cover letter. ation: 4 - 10 days; Exposure Duration: 0 - 4 o shwater); Water; Not determined by study au	days (0-96h) thors (i.e., chemical of ir	nterest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Di-ethylhexyl phthalate (DEHP) 1335277					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	Medium	DEHP was identified by name only in the report, but the CAS number was given on the cover sheet.		
	Metric 2:	Test Substance Source	High	The source is listed as "Hopkins and Williams D.K."		
	Metric 3:	Test Substance Purity	High	The purity is listed as "Analar", presumably this means analytical grade.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Negative controls were included in the study.		
	Metric 5:	Negative Control Response	High	Control mortality is reported as less than 4 percent.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated.		
Domain 3: Exposure Cł	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	This was a static renewal test carried out in 350-mL Pyrex glass dishes.		
	Metric 8:	Consistency of Exposure Administration	High	Exposure solution was renewed daily.		
	Metric 9:	Measurement of Test Substance Concentration	Low	Measurements were not conducted; only nominal concentrations were reported.		
	Metric 10:	Exposure Duration and Frequency	High	This was a 96-hour acute toxicity study with a 24-hour post-study depuration.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of concentrations tested is not legible in the scanned copy; the range is given as 1-10 mg/L. It appears that it may be 5 concentrations.		
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All concentrations exceeded the solubility limit of DEHP (0.003 mg/L).		
Domain 4: Test Organis	m					
0	Metric 13:	Test Organism Characteristics	Medium	G. pulex were collected from a tributary of the River Len at Holingbourne, Kent. Be- cause they were collected from the wild rather than reared in laboratory culture, there are minor reservations about the choice of test organisms.		
	Metric 14:	Acclimatization and Pretreatment	High	Acclimatization was conducted for 7 days.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	10 organisms were placed in each exposure vessel, with two replicates.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the organisms in question.		
		С	ontinued on next page .			

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1335277 Table: 1 of 1

		con	tinued from previou	is page		
Study Citation:	Oil,, Shell (1	Oil,, Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp Gammarus pulex (L) to				
Duration: Exposure Route, Media. Path:	toxicants wi Overall Dura Aquatic (free	toxicants with cover letter. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate	Arthropods; Gammarus pulex; Not Applicable	e (e.g., fungi or algae	studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1335277	1335277				
Domain	Domain Metric Rating Comments					
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed by observing appendage movement for 15 seconds.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed simultaneously and in the same way among all study groups.		
		Assessment				
Domain 6: Confoundin	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	LC50 values were calculated by probit analysis.		
	Metric 22:	Reporting of Data	High	Data for all outcomes were reported.		
	Metric 23.	Explanation of Unexpected Outcomes	High	There were no unexplained outcomes.		

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route,	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental Contamination and Toxicology 46(1):159-166. Overall Duration: > 21 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Behavioral Di-ethylhexy 732821	Arthropods; <i>Gammarus pulex</i> ; Adult /l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		C C			
	Metric 1:	Test Substance Identity	Medium	The chemical was only identified as DEHP, a phthalate ester.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
	Weute 7.	Preparation	Low	test concentrations. There was some concern over using plexiglass tanks with phthalates.		
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions regarding quality checks are likely to have an impact on results.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of the exposure was reported, but steady state or time dependent results were not reported.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only two exposure levels were used. This is not sufficient to obtain a dose response relationship.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4: Test Organis	Motria 12	Test Organism Characteristics	Madium	There are minor reconnections about the course of toot operations		
	Metric 13:	A colimatization and Protreatment	Lich	All protections about the source of test organisms.		
	Metric 14.	Conditions	nign	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Replicates were not used or reported.		
		Repleates per Group				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
		Conti	nued on next pa			

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Diethylhexyl Phthalate

		contin	ued from previo	ous page			
Study Citation:	Thurén, A., V	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental					
	Contamination	Contamination and Toxicology 46(1):159-166.					
Duration:	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Gammarus pulex; Adult						
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	732821	•					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Medium	Some details regarding the execution of the study protocol for outcome assessment were			
		Assessment		not reported.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	The results were highly variable with no clear trends.			
Additional Comments:	None						

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Thurén, A.,	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental						
Duration:	Overall Dura	on and Toxicology $46(1)$:159-166. ation: > 21 days; Exposure Duration: 4 - 10	days					
Exposure Route,	Aquatic (free	quatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taya Species Age:	Invertebrate	Arthropods: Gammarus pular: Adult						
Health Outcome:	ADME (biot	(ransformation)						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	732821	-						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Medium	The chemical was only identified as DEHP, a phthalate ester.				
	Metric 2:	Test Substance Source	Low	The source was not reported.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Demein 2. Ernerum Ch								
Domain 5: Exposure Ch	Matria 7	Environmental Sautana / Taat Madia	Ι					
	Metric 7:	Preparation	Low	test concentrations. There was some concern over using plexiglass tanks with phthalates.				
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions regarding quality checks are likely to have an impact on results.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of the exposure was reported, but steady state or time dependent results were not reported.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only two exposure levels were used. This is not sufficient to obtain a dose response relationship.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
D								
Domain 4: Test Organis	m							
	Metric 13:	lest Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.				
	Metric 14:	Conditions	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	Replicates were not used or reported.				
		et and the second						
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported, and water concentra- tions were not used to assess accumulation.				
	Continued on next page							

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental Contamination and Toxicology 46(1):159-166. **Duration:** Overall Duration: > 21 days; Exposure Duration: 4 - 10 days Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Gammarus pulex; Adult **Health Outcome:** ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 732821 Domain Metric Rating Comments Metric 18: Consistency of Outcome Medium Details regarding the execution of the study protocol for outcome assessment were not reported. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low The study did not provide enough information to allow a comparison of environmental Design and Procedures conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition. Domain 7: Data Presentation and Analysis Statistical Methods Metric 21: Low Statistical analysis was not conducted, which is typical for BCF assessments. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Explanation of Unexpected Outcomes Metric 23: High There were no unexpected outcomes. Additional Comments: None Medium **Overall Quality Determination**

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HERO ID: 732821 Table: 3 of 3

Study Citation: Duration: Exposure Route, Media, Path:	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental Contamination and Toxicology 46(1):159-166. Overall Duration: > 21 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 732821	Arthropods; <i>Gammarus pulex</i> ; Adult l phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Matria 1:	Tast Substance Identity	Madium	The chamical was only identified as DEUD a phthelate actor		
	Metric 2:	Test Substance Source	Low	The course was not reported		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
		•		- · ·		
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent pegative control		
	Metric 5:	Negative Control Response	Medium	Authors only reported that mortality was the same for control and treated organisms		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. There was some concern over using plexiglass tanks with phthalates.		
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions regarding quality checks are likely to have an impact on results.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only two exposure levels were used. This is not sufficient to obtain a dose response relationship.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4: Test Organic	m					
Domain +. Test Organis	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of test organisms.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Conditions Number of Organisms and	Low	Replicates were not used or reported.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
2 shain 5. Guteome Als	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.		
		(Continued on next page			

Diethylhexyl Phthalate

		cor	ntinued from previous	page		
Study Citation:	Thurén, A., V	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental				
	Contamination and Toxicology 46(1):159-166.					
Duration:	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Gammarus pulex; Adult				
Health Outcome:	Mortality	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	732821					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A	A. (1982). Significance of interfaces in the dis	stribution and metabolisr	n of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.					
Duration	Environmental Pollution 27(4):263-274. Overall Duration: > 21 days; Exposure Duration: > 21 days								
Exposure Route	Aquatic (free	retain Duration. > 21 days, Exposure Duration. > 21 days relation (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)							
Media. Path:	riquitie (iie)	require (neshwater), water, not determined by study autions (ne., enemiear of interest in exposure water, but anable to determine exact uptake route)							
Taxa, Species, Age:	Invertebrate:	; Arthropods; <i>Gammarus pulex</i> ; Not Applical	ble (e.g., fungi or algae s	tudies) or Not Reported					
Health Outcome:	ADME (biot	transformation)							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)							
HERO ID:	59542								
Domain		Metric	Rating	Comments					
Domain 1: Test Substanc	e								
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.					
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.					
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.					
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.					
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.					
Domain 3: Exposure Cha	aracterization								
	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.					
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.					
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).					
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.					
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.					
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than its approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.					
Domain 4: Test Organism	n								
	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.					
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment conditions.					
	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.					
		Replicates per Group							
Domain 5: Outcome Asso	essment								

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		cor	ntinued from previou	s page	
Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 da	iys		
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate;	; Arthropods; Gammarus pulex; Not Applicabl	le (e.g., fungi or algae	studies) or Not Reported	
Health Outcome:	ADME (biotransformation)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)			
HERO ID:	59542				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish was not well described.	
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of the 14C DEHP in the tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).	
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 140 DEHP.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test	Low	Multiple organisms were loaded into the same experimental tank could affect 14C DEHP untake by each organism.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.	
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.	
Additional Comments:	This study of mesocosm. (may not be a DEHP + me	collected 13 organisms (fish, invertebrates, a One tank containing the organisms was compl acutely harmful to fish." The chosen concentri tabolites, the metabolites being phthalic acid a	nd plants) from unpo eted. Fish were separa ration was known to b nd phthalic anhydride	Illuted streams, as well as the collection of water and sediment for the ted in the tank. Toxicity was addressed as "the results indicate that DEHP be below that of which would cause acute toxicity. The authors measured	

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A	A. (1982). Significance of interfaces in the dis	stribution and metabolism	n of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.					
D (1	Environmen	tal Pollution 27(4):263-274.							
Duration: Exposure Poute	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	lays thora (i.a., ahamiaal of in	starast in avnosura water, but unable to determine avest untake route)					
Exposure Route, Media Path.	Aquatic (fre	rquare (reshwater), water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake foute)							
Taxa Species Age	Invertebrate	Invertebrate: Worms (e.g. Annelids Nematodes): <i>Helobdella sn</i> : Not Applicable (e.g. fungi or algae studies) or Not Reported							
Health Outcome:	ADME (bio	ADME (biotransformation)							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)							
HERO ID:	59542								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.					
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.					
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.					
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.					
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.					
Domain 3: Exposure Ch	aracterization								
	Metric 7:	Experimental System/Test Media	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.					
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.					
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).					
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.					
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.					
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solu- bility in water (0.3 mg/L). Use of sectors as a solvent may increase solubility disblue					
				binty in water (0.5 mg/L). Use of acetone as a solvent may increase solubility signify.					
Domain 4: Test Organisi	n								
	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.					
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.					
	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.					

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		cont	tinued from previou	s page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Sodergren, A Environmen Overall Dura Aquatic (frea Invertebrate; ADME (biot Di-ethylhexy 59542	A. (1982). Significance of interfaces in the districtal Pollution 27(4):263-274. ation: > 21 days; Exposure Duration: > 21 days shwater); Water; Not determined by study authors (e.g., Annelids, Nematodes); <i>Helobdel</i> transformation) yl phthalate (DEHP)	ibution and metabolis /s ors (i.e., chemical of //a sp.; Not Applicabl	sm of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. interest in exposure water, but unable to determine exact uptake route) e (e.g., fungi or algae studies) or Not Reported
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in the tissue of the organisms as well as the water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.
Domain 6: Confounding	y / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms were loaded into the same experimental tank could affect 14C DEHP uptake by each organism.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.
Additional Comments:	This study of mesocosm. (may not be a DEHP + me	collected 13 organisms (fish, invertebrates, an One tank containing the organisms was comple acutely harmful to fish." The chosen concentra tabolites, the metabolites being phthalic acid an	d plants) from unpo ted. Fish were separa ation was known to b ad phthalic anhydride	lluted streams, as well as the collection of water and sediment for the ted in the tank. Toxicity was addressed as "the results indicate that DEHP e below that of which would cause acute toxicity. The authors measured .

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Jr, Mayer, F	F., Sanders, H. O., Walsh, D. F. (1973). To	oxicity, residue dynamics	s, and reproductive effects of phthalate esters in aquatic invertebrates.
Duration	Environmen	tal Research 6(1):84-90.	dava	
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)
Media, Path:	i iquaite (ii e			
Taxa, Species, Age:	Invertebrate;	; Arthropods; Hexagenia bilineata; Not Appl	icable (e.g., fungi or alga	e studies) or Not Reported
Health Outcome:	ADME (biot	transformation)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	1334040			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	Metric 1:	Test Substance Identity	High	The chemical was identified by name
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design	Metric 4.	Negative Controls	Uninformative	A concurrent negative control group was not included or reported
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization		-	
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Low	Few details of the exposure administration were reported, and exposures were adminis- tered consistently across study groups.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Low	Only one treatment was reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4. Test Organis	m			
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and	Low	The number of test replicates was not reported.
		Replicates per Group		
Domain 5: Outcome As	sessment			
2 ontain 5, Outcome 115	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.
		C	ontinued on next page .	

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Diethylhexyl Phthalate

		CO	ntinued from previous	s page	
Study Citation:	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. Environmental Research 6(1):84-90.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10	days		
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Invertebrate; Arthropods; Hexagenia bilineata; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	ADME (biot	ADME (biotransformation)			
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)			
HERO ID:	1334646				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for outcome assessment were	
		Assessment		provided.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.	
Domain 7: Data Present	tation and Anal	vsis			
	Metric 21:	Statistical Methods	Low	Statistical analysis was not performed.	
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for the sampling period.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None				
Overall Quali	ty Detern	nination	Uninformativ	'e	

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Call, D. J., C	Cox, D. A., Geiger, D. L., Genisot, K. I., Ma	arkee, T. P., B	rooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,
	Parkerton, T	F., Reiley, M. C., Ankley, G. T., Mount,	D. R. (2001).	An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment
	exposures. E	Environmental Toxicology and Chemistry 2	20(8):1805-18	315.
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days	
Exposure Route,	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake			
Media, Path:				
Taxa, Species, Age:	Invertebrate;	Arthropods; Hyalella azteca; Not Applica	ıble (e.g., fun	gi or algae studies) or Not Reported
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	679311			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. Further details, such as CASRN, were provided in Call et al 2001.
	Metric 2:	Test Substance Source	High	The source of chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	Purity was identified as 99% in the cited reference Call et al 2001.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	The biological response of the control group was appropriate, as shown in Table 5.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.
Domain 3: Exposure Ch	naracterization			
ľ	Metric 7:	Experimental System/Test Media	High	Methods of sediment collection and preparation (including chemical addition) and addi- tion of sediment to test beakers were described in detail
	Metric 8.	Consistency of Exposure	High	Exposure consistency was reported and consistent
	metile 0.	Administration	mgn	Exposure consistency was reported and consistent.
	Metric 9:	Measurement of Test Substance	High	Concentrations were measured using HPLC as described in the methods and referenced
		Concentration		in Call et al 2001.
	Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed cited methods (EPA, 1994).
	Metric 11:	Number of Exposure Groups/	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to exposure was not reported.
	Metric 15.	Conditions Number of Organisms and	Medium	Tests with DEHP DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with
	wicult 13.	Replicates per Group	wiculuill	10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.

Diethylhexyl Phthalate

		conti	nued from p	revious page	
Study Citation: Duration:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stu	dy authors (i	.e., chemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Invertebrate;	Arthropods; Hyalella azteca; Not Applica	ble (e.g., fun	gi or algae studies) or Not Reported	
Health Outcome:	Mortality				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	679311				
Domain		Metric	Rating	Comments	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10, and conductivity was measured on days 1 and 9.	
	Metric 17:	Outcome Assessment Methodology	Medium	The survivor count was determined after the 10 day exposure, but it was not reported as percent mortality.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups that could influence outcome assessment.	
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were re- ported for each study group, and there were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Present	ation and Anal	veis			
Domain 7. Data i lesen	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized usingthe trimmed Spearman–Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dun- nett'sprocedure using a SigmaStatt Program.	
	Metric 22:	Reporting of Data	Medium	Data were reported in Table 5, however results were pooled among replicates.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.	
Additional Comments:	None				
Overall Qualit	tv Deterr	nination	High		

HERO ID: 679311 Table: 2 of 2

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days		
Exposure Route,	Aquatic (fre	shwater); Sediment; Not determined by stu	dy authors (i	.e., chemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:	•	•	•		
Taxa, Species, Age:	Invertebrate	; Arthropods; Hyalella azteca; Not Applica	able (e.g., fun	gi or algae studies) or Not Reported	
Health Outcome:	Developmen	nt/Growth			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)			
HERO ID:	679311				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. Further details, such as CASRN, were provided in Call et al 2001.	
	Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).	
	Metric 3:	Test Substance Purity	High	Purity was identified as 99% in the cited reference Call et al 2001.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.	
	Metric 5:	Negative Control Response	High	The biological response of the control group was appropriate as shown in Table 5.	
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.	
Domain 3: Exposure Ch	aracterization				
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	Methods of sediment collection and preparation (including chemical addition) and addi-	
		Preparation	U	tion of sediment to test beakers were described in detail.	
	Metric 8:	Consistency of Exposure	High	Exposure consistency was reported and consistent.	
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods and referenced in Call et al 2001.	
	Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed the cited methods (EPA, 1994).	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organis	m		_		
	Metric 13:	Iest Organism Characteristics	Low	The source of the test organisms was not provided.	
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of the test organisms prior to exposure was not reported.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker.	

Domain 5: Outcome Assessment

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May 2025 Environmental Hazard Evaluation

Diethylhexyl Phthalate

		conti	nued from p	revious page	
Study Citation: Duration:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stu	dy authors (i	.e., chemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Invertebrate;	; Arthropods; Hyalella azteca; Not Applica	ble (e.g., fun	gi or algae studies) or Not Reported	
Health Outcome:	Development/Growth				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	679311				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10, and the conductivity was measured on days 1 and 9.	
	Metric 17:	Outcome Assessment Methodology	High	The sediment was sieved. Survivors were then collected, dried, and weighed.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups that could influence outcome assessment.	
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were re- ported for each study group, and there were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized usingthe trimmed Spearman–Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dun- nett'sprocedure using a SigmaStatt Program.	
	Metric 22:	Reporting of Data	High	Treatment and control data were reported in Table 5. Results were represented as aver- age dry weight per individual.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.	
Additional Comments:	None				
Overall Qualit	ty Detern	nination	High		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:Call, D. J., Markee, T. P., Geiger, D. L., B F., Reiley, M. C., Ankley, G. T., Mount, D Environmental Toxicology and Chemistry 2Duration:Overall Duration: 4 - 10 days; Exposure Du Aquatic (freshwater); Water; Not determined Media, Path:Taxa, Species, Age:Invertebrate; Arthropods; Hyalella azteca; J Health Outcome:Methal:Di-ethylhexyl phthalate (DEHP)HERO ID:679312DomainMetricDomain 1: Test SubstanceMetric 1: Metric 2:Metric 2:Test Substance FourtyDomain 2: Test DesignMetric 4: Metric 5: Negative Controls Metric 6:Domain 3: Exposure Characterization Metric 7:Metric 7: Experimental System/Test M Preparation	rooke, L. T., Vandeven . R. (2001). An assess 0(8):1798-1804. ration: 4 - 10 days d by study authors (i.e., uvenile Rating	ter, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. nent of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. chemical of interest in exposure water, but unable to determine exact uptake route)
Duration:Overall Duration: 4 - 10 days; Exposure DuExposure Route, Media, Path:Aquatic (freshwater); Water; Not determinedTaxa, Species, Age:Invertebrate; Arthropods; Hyalella azteca; JHealth Outcome: MortalityMortalityChemical: DomainDi-ethylhexyl phthalate (DEHP)HERO ID:679312DomainMetricDomain 1: Test Substance Metric 2:Test Substance Identity Metric 2:Metric 3:Test Substance PurityDomain 2: Test DesignMetric 4: Metric 5: Negative Controls Metric 6:Domain 3: Exposure Characterization Metric 7:Metric 7: Experimental System/Test M Preparation	ration: 4 - 10 days d by study authors (i.e., uvenile Rating	chemical of interest in exposure water, but unable to determine exact uptake route)
Exposure Route, Media, Path:Aquatic (freshwater); Water; Not determined Media, Path:Taxa, Species, Age: Health Outcome: Chemical: DomainInvertebrate; Arthropods; Hyalella azteca; J Mortality Di-ethylhexyl phthalate (DEHP) HERO ID:HERO ID:679312DomainMetricDomain 1: Test Substance Metric 2: 	d by study authors (i.e., uvenile Rating	chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path: Invertebrate; Arthropods; Hyalella azteca; J Taxa, Species, Age: Invertebrate; Arthropods; Hyalella azteca; J Health Outcome: Mortality Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 679312 Domain Metric Domain 1: Test Substance Metric 1: Metric 2: Test Substance Identity Metric 3: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	uvenile Rating	
Taxa, Species, Age: Health Outcome: Chemical: DomainInvertebrate; Arthropods; Hyalella azteca; J Mortality Di-ethylhexyl phthalate (DEHP) HERO ID:HERO ID:679312DomainMetricDomain 1: Test Substance Metric 2:Test Substance Identity Metric 2: Test Substance SourceMetric 3:Test Substance PurityDomain 2: Test Design 	uvenile Rating	
Health Outcome: Mortality Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 679312 Domain Metric Domain 1: Test Substance Metric 1: Metric 2: Test Substance Identity Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M	Rating	
Chemical: HERO ID: Di-ethylhexyl phthalate (DEHP) 679312 679312 Domain Metric Domain 1: Test Substance Metric 1: Metric 2: Metric 2: Metric 3: Test Substance Identity Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Metric 5: Metric 6: Randomized Allocation Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	Rating	
HERO ID: 679312 Domain Metric Domain 1: Test Substance Metric 1: Metric 2: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	Rating	
Domain Metric Domain 1: Test Substance Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 2: Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M	Rating	
Domain 1: Test Substance Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation		Comments
Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation		
Metric 2: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	High	The chemical was identified by name. No CASRN or structure were provided.
Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Metric 5: Negative Controls Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	High	The source of the phthalate was Aldrich Chemical. The test substance identity was not analytically verified by the performing laboratory.
Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	High	The chemical purity was reported as $>98\%$.
Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation		
Metric 5: Negative Control Response Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	High	Study authors reported using an appropriate concurrent negative control group.
Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	High	The biological response of the negative control group was adequate.
Domain 3: Exposure Characterization Metric 7: Experimental System/Test M Preparation	Low	Researchers did not report how organisms were allocated to study groups.
Metric 7: Experimental System/Test M Preparation		
	ledia High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.
Metric 8: Consistency of Exposure	High	The exposures were administered consistently across study groups.
Metric 9: Measurement of Test Substan Concentration	nce High	Exposure concentrations were measured using appropriate analytical technologies and methods.
Metric 10: Exposure Duration and Frequ	uency Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.
Metric 11: Number of Exposure Groups Spacing of Exposure Levels	/ Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response.
Metric 12: Testing at or Below Solubilit	y Limit Medium	A subset of the exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism		
Metric 13: Test Organism Characteristic	es High	The test organisms were adequately described and were obtained from a reliable source.
Metric 14: Acclimatization and Pretreat	ment High	All pretreatment conditions were the same for control and exposed organisms.
Conditions Metric 15: Number of Organisms and Replicates per Group	-	The number of test organisms and replicates were reported and sufficient to characterize

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

Diethylhexyl Phthalate

		conti	nued from p	revious page
Study Citation:	Call, D. J., M F., Reiley, M Environment	Markee, T. P., Geiger, D. L., Brooke, L. T I. C., Ankley, G. T., Mount, D. R. (2001). tal Toxicology and Chemistry 20(8):1798-1	., Vandevent An assessn 1804.	er, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. nent of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures.
Duration:	Overall Dura	tion: 4 - 10 days; Exposure Duration: 4 - 1	l0 days	
Exposure Route, Media, Path:	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Hyalella azteca; Juvenile		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	679312			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.
	Metric 18:	Consistency of Outcome	High	The outcomes were assessed consistently across study groups.
		Assessment		
Domain 6: Confounding	g / Variable Coi	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.
Additional Comments:	None			
Overall Quali	ty Detern	nination	High	

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Duration: C Exposure Route, Media, Path:	Environmenta Overall Dura Aquatic (fres	al Pollution 27(4):263-274. tion: > 21 days; Exposure Duration: > 21 d hwater); Water; Not determined by study aut	ays thors (i.e., chemical of in	
Exposure Route, Media, Path:	Aquatic (fres	hwater); Water; Not determined by study aut	ays thora (i.e., chamical of in	
Media, Path:	Invertebrate	invator), water, not determined by study ad		terest in exposure water, but unable to determine exact uptake route)
	Invertebrate		litors (i.e., enemieur or in	
Taxa, Species, Age:	mvenceorace,	Arthropods; Limnephilus sp; Larvae		
Health Outcome:	ADME (biotr	ransformation)		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	59542			
Domain		Metric	Rating	Comments
Domain 1: Test Substance	Madula 1.	Trat Caleston - Identita	Madin	
1	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.
1	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.
]	Metric 3:	Test Substance Purity	High	The purity was 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.
]	Metric 5:	Negative Control Response	N/A	No negative control was group reported.
]	Metric 6:	Randomized Allocation	Low	No random allocation was reported.
Domain 3: Exposure Chara	acterization			
l	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.
1	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.
]	Metric 9:	Measurement of Test Substance	High	The test substance was measured by TLC and liquid scintillation. Final concentrations
		Concentration		of sediment, glass walls, surface microlayer, suspended material, and groups of organ-
				isms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride)
]	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.
1	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.
		Spacing of Exposure Levels		
1	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L, which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.
Domain 4: Test Organism				
]	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.
1	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.
1	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.
		Replicates per Group		- •
Domain 5: Outcome Assos	ssment			

Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Sodergren, A Environment Overall Dura Aquatic (fres Invertebrate; ADME (biotr Di-ethylhexy 59542	(1982). Significance of interfaces in the dist al Pollution 27(4):263-274. tion: > 21 days; Exposure Duration: > 21 da hwater); Water; Not determined by study auth Arthropods; <i>Limnephilus sp</i> ; Larvae ransformation) l phthalate (DEHP)	ribution and metabolis ys tors (i.e., chemical of it	m of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.
Domain 6: Confounding	/ Variable Con	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP uptake by each organism.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Present	ation and Analy	ysis		
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.
Additional Comments:	This study c mesocosm. C may not be a DEHP + met	ollected 13 organisms (fish, invertebrates, a One tank containing the organisms was comple cutely harmful to fish." The chosen concentr abolites, the metabolites being phthalic acid a	nd plants) from unpol eted. Fish were separat ration was known to be and phthalic anhydride.	luted streams, as well as the collection of water and sediment for the ed in the tank. Toxicity was addressed as "the results indicate that DEHP below that of which would cause acute toxicity. The authors measured

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Call, D. J., F., Reiley, M Environmen	Markee, T. P., Geiger, D. L., Brooke, L. A. C., Ankley, G. T., Mount, D. R. (2001 tal Toxicology and Chemistry 20(8):1798.	T., Vandevent). An assessn -1804.	er, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. nent of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures.
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days	
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	1		× ,	
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Lun	nbriculus vari	egatus; Adult
Health Outcome:	Mortality			
Chemical:	Di-ethylhex	yl phthalate (DEHP)		
HERO ID:	679312			
Domain		Metric	Rating	Comments
Domain 1: Test Substar	ice			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.
	Metric 2:	Test Substance Source	High	The source of the phthalate was Aldrich Chemical. The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as $>98\%$.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Cl	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	The exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organis	sm			
J	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

		conti	nued from p	revious page
Study Citation:	Call, D. J., I F., Reiley, M Environment	Markee, T. P., Geiger, D. L., Brooke, L. T I. C., Ankley, G. T., Mount, D. R. (2001) tal Toxicology and Chemistry 20(8):1798-	T., Vandevent . An assessm 1804.	er, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. nent of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures.
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days	
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	-			
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Lum	briculus vari	egatus; Adult
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	679312			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment		
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.
Additional Comments:	None			
Overall Qualit	ty Detern	nination	High	

Study Citation:	Sung, H. H.,	Kao, W. Y., Su, Y. J. (2003). Effects and	toxicity of phtha	late esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii.
Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Aquatic Toxi Overall Dura Aquatic (free uptake route) Invertebrate:	(tion: 0 - 4 days (0-96h); Exposure Duration shwater); Cell Culture Media; Not determi) Arthropods: <i>Macrobrachium rosenbergii</i> : 1	n: 0 - 4 days (0-9 ined by study aut	6h) thors (i.e., chemical of interest in exposure water, but unable to determine exact
Health Outcome: Chemical: HERO ID:	Mechanistic- Di-ethylhexy 789598	Cell signaling/function	(Contripplication)	.g., rangi of algae statico) of root reported
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by correct nomenclature and chemical structure. The CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source of DEHP was Aldrich Chem. Co.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	The biological responses of the controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors reported that the phthalate stocks were separately dissolved in acetone and diluted with M-199 (hemocyte-culture medium) to a concentration of 1000 mg/ml. They also reported the final concentration once the phthalates were added to the hemocyte suspension. However, the authors did not report the acetone concentration employed.
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance	Low	The exposure concentrations were not reported/measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate to assess cell death (necrosis and apoptosis) and cell morphology impacts in the hemocytes.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentration (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The concentrations were below solubility limit as reported. But, the authors did not report the concentration of acetone used to dissolve the phthalate, and they did not measure the phthalate concentration.

Domain 4: Test Organism

Diethylhexyl Phthalate

		con	tinued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Sung, H. H., Aquatic Tox Overall Dura Aquatic (fre- uptake route Invertebrate; Mechanistic- Di-ethylhexy	Kao, W. Y., Su, Y. J. (2003). Effects an icology 64(1):25-37. ition: 0 - 4 days (0-96h); Exposure Durati shwater); Cell Culture Media; Not deterr) Arthropods; <i>Macrobrachium rosenbergii</i> -Cell signaling/function /1 phthalate (DEHP)	d toxicity of phtha on: 0 - 4 days (0-9 nined by study aut ; Not Applicable (e	late esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. 6h) hors (i.e., chemical of interest in exposure water, but unable to determine exact e.g., fungi or algae studies) or Not Reported
HERO ID:	789598			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (freshwater prawn) were obtained from a market, and the hemolymph drawn from these test organisms was then used to isolate the hemocytes employed in the in vitro exposure and assays. Information on the size/age of the prawns was not provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 C for 3 days prior to experiments, and stocking densities were main-tained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect the immune system. The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.
	Metric 15:	Number of Organisms and Replicates per Group	Low	This was an in vitro study to determine impacts on hemocytes (immune cells) measured by cell death (necrosis and apoptosis) and cell morphology. The number of organisms used (5-10) to collect a diverse pool of hemocytes and cell suspensions was reported. However, authors offered insufficient details on, for example, the number of replicates examined by gel electrophoresis or the number of sections and replicate fields for the electron microscopy assessment.
Domain 5: Outcome A	ssessment			

				examined by gel electrophoresis or the number of sections and replicate fields for the electron microscopy assessment.
Domain 5: Outcome As	ssessment			
	Metric 16:	Adequacy of Test Conditions	High	This was an in vitro exposure study to determine impacts on hemocytes measured by cell death (necrosis and apoptosis) and cell morphology. In vitro exposure conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies using hemocytes [the annexin assay, gel elec- trophoresis, transmission electron microscopy] were reported and appropriate for the outcomes of interest in hemocytes [cell death by necrosis and apoptosis and cell mor- phology changes].
	Metric 18:	Consistency of Outcome Assessment	High	Details of the in vitro assays to examine hemocyte toxicity/viability were reported and assessed consistently across groups.
Domain 6: Confoundin	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared in adequate medium conditions and treated consistently.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Presen	tation and Anal	lysis		
	Metric 21:	Statistical Methods	Low	Statistical analysis was reported but not explained in detail.
	Metric 22:	Reporting of Data	High	Data were reported for each assay and for all treatment groups per assay. The summary of the effects in Table 1 reported the inhibition or enhancement concentration for each assay.
		Contin	nued on next p	age

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Diethylhexyl Phthalate

HERO ID: 789598 Table: 1 of 2

		continu	ed from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Sung, H. H. Aquatic Tox Overall Dura Aquatic (fre uptake route Invertebrate; Mechanistic Di-ethylexy	Kao, W. Y., Su, Y. J. (2003). Effects and to icology 64(1):25-37. ation: 0 - 4 days (0-96h); Exposure Duration: shwater); Cell Culture Media; Not determine Arthropods; <i>Macrobrachium rosenbergii</i> ; No -Cell signaling/function /l phthalate (DEHP)	oxicity of phth 0 - 4 days (0-9 ed by study au ot Applicable (alate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. 6h) thors (i.e., chemical of interest in exposure water, but unable to determine exact e.g., fungi or algae studies) or Not Reported
HERO ID:	/89598			
Domain	Metric 23:	Metric Explanation of Unexpected Outcomes	Low	Comments Authors did not report SE, SD, CI or such variability details for the cell death (necrosis and apoptosis) or the cell morphology data.
Additional Comments:	This study w rosenbergii) viability assa (a measure o functions), a toxicity/viab microscopy.	vas an in vitro experiment where hemocytes (were exposed to BBP, DBP, DEHP, or DCHP ays: (1) Nonspecific cell-mediated immune de f the initial procedures of either phagocytosis nd nitroblue tetrazolium solution (NBT) assa ility assays included detection of cell death vi This form was used to evaluate hemocyte tox	immune cells) ? Endpoints en efense response s or encapsulat y to determine ia necrosis, det cicity/viability	isolated from the hemolymph of 5 to 10 giant freshwater prawn (Macrobrachium compassed nonspecific cell-mediated immune function assays as well as hemocyte assays included determination of hemocytic adhesion and pseudopodia formation on), phenoloxidase activity assay (a measure of pathogen recognition and defense superoxide production (a measure of highly microbicidal activity); (2) Hemocyte ection of cell death via apoptosis, and impacts on cellular morphology assessed by due to DEHP.

Overall Quality Determination

Medium

Study Citation:	Sung, H. H.,	Kao, W. Y., Su, Y. J. (2003). Effects and t	toxicity of phtha	late esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii.
Duration	Aquatic Toxi	cology 64(1):25-37.	• 0 4 days (0 0	Z L\
Duration: Exposure Doute	Aquatic (free	human (0-4 days (0-901); Exposure Duration	0 - 4 days(0-9)	UII) hore (i.e., chamical of interact in exposure water, but unable to determine exact
Exposure Noute, Modia Dath:	Aquatic (fres	inwater), Cell Culture Media, Not determin	led by study aut	nois (i.e., chemical of interest in exposure water, but unable to determine exact
Tava Spacias Aga	Invertebrate:	Arthropods: Macrobrachium rosanharaii: N	Iot Applicable (e	a funci or algae studies) or Not Reported
Health Outcome	Mechanistic-	Cell signaling/function	(of Applicable (e	.g., rungi of algae studies) of Not Reported
Chemical.	Di-ethylhexy	l phthalate (DFHP)		
HERO ID:	789598			
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by correct nomenclature and chemical structure. The CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source of DEHP was Aldrich Chem. Co.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	The biological responses of the controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure Cha	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors reported that the phthalate stocks were separately dissolved in acetone and diluted with M-199 (hemocyte-culture medium) to a concentration of 1000 mg/ml. They also reported the final concentration once the phthalates were added to the hemocyte suspension. However, the authors did not report the acetone concentration employed.
	Metric 8:	Consistency of Exposure	High	The exposure was consistent.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not reported/measured.
	N 10	Concentration		
	Metric 10:	Exposure Duration and Frequency	High	The exposure time was appropriate.
	Metric 11:	Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentrations (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or in only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	All concentrations were below the solubility limit as reported. But, the authors did not report the concentration of acetone used to dissolve the phthalate, and they did not measure the phthalate concentration.
Domain 4: Test Organisr	n			
	Metric 13:	Test Organism Characteristics	Medium	Test organisms (freshwater prawn) were obtained from a market, and hemolymph drawn from these test organisms was then used to isolate the hemocytes employed in the in vitro exposure and assays. Information on the size/age of prawn was not provided.
		Contir	nued on next pa	ge

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Diethylhexyl Phthalate

		contin	nued from previ	ous page
Study Citation:	Sung, H. H., Aquatic Tox	Kao, W. Y., Su, Y. J. (2003). Effects and icology 64(1):25-37.	toxicity of phtha	late esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii.
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)
Exposure Route,	Aquatic (free	shwater); Cell Culture Media; Not determi	ined by study au	thors (i.e., chemical of interest in exposure water, but unable to determine exact
Media, Path:	uptake route)		
Taxa, Species, Age:	Invertebrate;	Arthropods; Macrobrachium rosenbergii; 1	Not Applicable (e	e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic	Cell signaling/function		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	789598			
Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 C for 3 days prior to experiments, and stocking densities were main-tained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect immune function (i.e., stress can modulate immune responses (cell-mediated and humoral). The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	This was an in vitro study to determine impacts on nonspecific cell-mediated immune defense responses. The number of organisms used (5-10) to collect a diverse pool of hemocytes, number of cells and cell suspensions, number of well-plates, and number of replicate fields for microscopic counts were sufficient.
Domain 5: Outcome As	ssessment			
	Metric 16:	Adequacy of Test Conditions	High	This was an in vitro exposure study to determine impacts on nonspecific cell-mediated immune defense responses. In vitro exposure conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies [determination of hemocytic adhesion and pseu-

				dopodia formation assay, the phenoloxidase activity assay, and the nitroblue tetrazolium assay] were reported and appropriate for the outcomes of interest [phagocytosis and encapsulation activity, pathogen recognition, and superoxide activity as a measure of microbicidal activity].
	Metric 18:	Consistency of Outcome	High	Details of the immune function of in vitro assays were reported and assessed consis-
		Assessment		tently across groups.
		1 bbessment		
Domain 6: Confoundi	ng / Variable Co	ntrol		
Domain 6: Confoundi	ng / Variable Co Metric 19:	ntrol Confounding Variables in Test	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared
Domain 6: Confoundi	ng / Variable Co Metric 19:	ntrol Confounding Variables in Test Design and Procedures	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared in adequate medium conditions and treated consistently across treatment groups.

Domain 7: Data Presentation and An	alysis		
Metric 21:	Statistical Methods	Low	Statistical analysis was reported but not explained in detail.
Metric 22:	Reporting of Data	High	Data were reported for each assay and for all treatment groups per assay. The summary of the effects in Table 1 reported the inhibition or enhancement concentration for each assay.
Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean for the nonspecific immune response data: hemocyte adhesion and pseudopodia formation, phenoloxidase activity, and super- oxide production (Figures 2 and 3).

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

continued from previous page						
Study Citation:	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). H Aquatic Toxicology 64(1):25-37.	Effects and toxicity of phthalate esters to	hemocytes of giant freshwater prawn, Macrobrachium rosenbergii.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Media, Path:	uptake route)					
Taxa, Species, Age:	Invertebrate; Arthropods; Macrobrachium rosenbergii; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-Cell signaling/function					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	789598					
Domain	Metric	Rating	Comments			
Additional Comments:	This study was an in vitro experiment where hemocytes (immune cells) isolated from the hemolymph of 5 to 10 giant freshwater prawn (Macrobrachium					
	rosenbergii) were exposed to BBP, DBP, DEHP, or DCHP. Endpoints encompassed nonspecific cell-mediated immune function assays as well as hemocyte					
	viability assays: (1) Nonspecific cell-mediated immune defense response assays included determination of hemocytic adhesion and pseudopodia formation					
(a measure of the initial procedures of either phagocytosis or encapsulation), phenoloxidase activity assay (a measure of pathogen recognition and defense						
functions), and nitroblue tetrazolium solution (NBT) assay to determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide production (a measure of highly microbicidal activity); (2) Hemocratical determine superoxide productical						
toxicity/viability assays included detection of cell death via necrosis, detection of cell death via apoptosis, and impacts on cellular morphology assessed b						
	microscopy. This form was used to evaluate impacts to the nonspecific cell-mediated immune defense responses due to DEHP.					

Overall Quality Determination

Medium
Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic						
	organisms. E	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Durati	on: 0 - 4 days	(0-96h)			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (1.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	T . 1 .						
Taxa, Species, Age:	Invertebrate;	Arthropods; Paratanytarsus parthenogen	<i>etica</i> ; Not Ap	plicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
UEDO ID.	Di-ethylnexy	/I phthalate (DEHP)					
	1321990						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	— — — — — — — — — — — — — — — — — — —	-				
	Metric 1:	Test Substance Identity	Low	The rest substance nomenclature was reported without a CASRN.			
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number not provided. No analytical data was reported.			
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A negative control was reported.			
	Metric 5:	Negative Control Response	High	The control response was acceptable.			
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7	Experimental System/Test Media	Medium	The experimental system was well described. However, headspace or measures taken to			
	Wette 7.	Preparation	Wiedium	prevent volatilization were not reported.			
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.			
	Metric 9:	Administration Measurement of Test Substance	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of test. In			
		Concentration		static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test			
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test.			
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.			
		Spacing of Exposure Levels	8				
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.			
Domain 4: Test Organis	m		-				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.			
	Metric 15.	Conditions Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test			
		Replicates per Group	moutuill	vessel.			
		T					
Domain 5: Outcome Ass	sessment						

Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic			
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-15	574.			
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Paratanytarsus parthenogene	<i>tica</i> ; Not Ap	plicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.			
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.			
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent across groups.			
		Assessment					
Domain 6: Confounding	g / Variable Con	ntrol					
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.			
		Design and Procedures	e				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.			
Domain 7: Data Present	ation and Anal	vsis					
Domain 7: Data Present	Metric 21	Statistical Methods	High	Statistical methods were performed and described			
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported			
	Metric 22:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported			
	metric 23.	Explanation of Onexpected Outcomes	ingn				
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

Study Citation: Duration: Exposure Route, Media Path:	Monsanto, (1983). Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge Paratanytarsus parthenogenetica. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Paratanytarsus parthenogenetica; Larvae					
Health Outcome:	Mortality	1, , , , , , , , , , , , , , , , , , ,	,				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1335357	1335357					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name.			
	Metric 2:	Test Substance Source	High	The source of DEHP was R. H. Mills, Plasticizers Division, Monsanto, St. Louis, Mis- souri. Additional information such as the lot number was accounted for in the log book and in the paper. It was not verified analytically.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.			
	Metric 5:	Regarized Allocation	High	I ne biological responses of the negative control groups were adequate.			
	Metric 6.	Randomized Anocation	Medium	Researchers state that 10 midge were randomly assigned to each test vessel.			
Domain 3: Exposure Ch	aracterization						
I	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported, but minor inconsistencies in admin- istration of exposures among study groups were identified that are unlikely to have a substantial impact on results.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	A standard duration was used.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	The concentrations used in the study ranged from 0.62-10 mg/L; all exceeded the limit of solubility.			
Domain 4: Test Organis	m						
Domain +. Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
				•			
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.			
		Cont	inued on nex	t nage			
		Com					

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Monsanto, (1983). Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge Paratanytarsus parthenogenetica. **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route**, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Paratanytarsus parthenogenetica; Larvae **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1335357 Domain Metric Rating Comments Outcome Assessment Methodology Metric 17: High The outcome assessment methodology addressed the intended outcome of interest. Metric 18: Consistency of Outcome Low Details regarding the execution of the study protocol for outcome assessment were not Assessment reported. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Sufficient data were provided to conduct an independent statistical analysis. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes. Additional Comments: Please note there is a typo on Table 4.

Overall Quality Determination

Low

Study Citation: Duration:	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (fres	shwater); Water; Not determined by study	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; <i>Paratanytarsus parthenogenica</i> ; Larvae					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1316219						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by name and CASRN.			
	Metric 2:	Test Substance Source	High	The phthalate ester was received from General Electric Company.			
	Metric 3:	Test Substance Purity	High	The purity was reported as 100% active ingredient.			
Domain 2: Test Design							
-	Metric 4:	Negative Controls	High	Negative controls were used.			
	Metric 5:	Negative Control Response	High	Percent mortality for the controls was shown in Table 3.			
	Metric 6:	Randomized Allocation	Medium	Organisms were impartially distributed into the test vessels.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	Static test conditions were described in detail.			
		Preparation	8				
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported but limited (volumes of chemicals used to make stock solutions were not reported).			
	Metric 9:	Measurement of Test Substance Concentration	High	The test concentration was verified analytically as shown in Table 1 and described in Appendix I.			
	Metric 10:	Exposure Duration and Frequency	High	The duration was reported and adequate (mortality reported at 48 hr and 24 hr expo- sure).			
	Metric 11:	Number of Exposure Groups/	N/A	One concentration of DEHP was tested.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The concentration did not exceed the approximate solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Paratanytarsus parthenogenica was obtained from cultured stocks (EG&G Bionomics); age was reported as second or third instars.			
	Metric 14:	Acclimatization and Pretreatment	Medium	It was not specifically stated if the organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and	Medium	The number of replicates was acceptable with thirty total organisms, 10 per vessel.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described and reported, and they followed cited protocols.			
	Metric 17:	Outcome Assessment Methodology	Medium	There were limited details of how authors determined mortality of the test organisms.			
		Cont	inued on nev	t nage			
		Cont					

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Diethylhexyl Phthalate

... continued from previous page Bionomics, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424. **Study Citation: Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Paratanytarsus parthenogenica; Larvae **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1316219 Domain Metric Rating Comments Metric 18: Consistency of Outcome High Mortality assessment was conducted at 24 and 48 hr and appeared to be consistent among study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High No differences were reported. Design and Procedures Metric 20: Outcomes Unrelated to Exposure High Details regarding test organism attrition and outcomes unrelated to exposure (e.g., infection) were reported for each study group, and there were no differences among groups that could influence the outcome assessment. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low It was unclear how authors obtained an LC50 based on a limit test (method for estimating LC50 not well described). Metric 22: Reporting of Data High Mortality results were shown in Table 3. Metric 23: Explanation of Unexpected Outcomes Medium Outcomes were explained in the study. Additional Comments: Authors conducted dose response of DBP and limit tests for DEHP, DIDP, and DINP. Authors report percent morality in replicate groups at 24 and 48 hr exposure. LC50 values for DBP were reported. LC50 values for DEHP, DIDP, and DINP were also reported, but it was unclear how authors obtained (or estimated) LC50 values based on a limit test.

Overall Quality Determination High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Duration: 21 days Exposure Route, Wedia, Pati- Heath, Overall Duration: 21 days Exposure Route, Wedia, Pati- Heath, Overall Duration: 21 days Description: 21 days Domain ADME (biotransformation) Demain Metric Domain Metric Domain Metric Ormain 1: Test Substance High Metric 2: Test Substance Bource Metric 3: Test Substance Purity Metric 4: Negative Controls Uninformative No negative control group was reported. Metric 5: Negative Control Secontrol Resonace N/A No negative control group was reported. Metric 6: Randomized Allocation Low Jonain 3: Exposure Characterization Metric 6: Randomized Allocation Metric 10: Exposure Allocation Asing dwas watenouterin 20-liter make al 1.43	Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.				
Online Orient Distance Aquatic (Teshwater), Water: Not determine by study attrons (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media, Path: Taxa, Species, Age: Invertebrate: Domain Determine: Domain Metric 1: Test Substance Metric 2: Test Substance Metric 3: Test Substance Metric 4: Negative Control Response Metric 5: Test Substance Metric 6: Retric 6: Negative Control Response Metric 6: Negative Control Response Netric 6: Negative Control Response North 6: North 6: North 7: North 7: Metric 2: Test Substance Identity Metric 3: Test Substance Identity Metric 4: Negative Control Response N/A Not nactive response Metric 5: Re	Duration:	Environment	tal Pollution 27(4):263-274.	0.1/2		
Media, Parti: Intervebrate: Molluskis: Planorbis corneus: Not Applicable (e.g., fungi or algae studies) or Not Reported Tealth Outcome: ADME (biotransformation) Chemical: Di-ethylbexyl phalatae (DEHP) S9542 Domain Metric 1: Test Substance Metric 2: Test Substance Identity Medium CoASRN estructures vere provided Metric 2: Test Substance Runce High The source was identified as New England Nuclear. Metric 3: Test Substance Purity High The purity was 95.56. Domain 2: Test Substance Identity Medium Metric 4: Negative Controls Metric 6: Randomized Allocation No random allocation was reported. Metric 6: Randomized Allocation No random allocation was reported. Metric 6: Randomized Allocation No random allocation was reported. Metric 6: Randomized Allocation No random allocation was reported. Metric 7: Speprimental System/Test Media Medium Metric 9: Metric 10: Exposure Duration and Frequency High The 2-Metric 10: Exposure Duration and Prequency High The 2-Metric 10: Exposure Characteristics No econstructure N	Exposure Route.	Aquatic (free	shwater): Water: Not determined by study au	ays thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)	
Data, Species, Age: Invertebrate: Molluskis: Planobis corneus; Not Applicable (e.g., fungi or algae studies) or Not Reported Health Outcome ADDER (foiloransformation) Domain 0) Chemical: Dost-alty/thexyl pithalaie (DFHP) Brain Dir Systa Domain 1: Test Substance Metric 2: Metric 2: Test Substance Identity Metric 3: Test Substance Network Metric 3: Test Substance Purity Domain 1: Test Substance Purity High The source was identified as New England Nuclear. Metric 3: Test Substance Purity Metric 5: Negative Controls No negative control group was reported. Metric 6: Randomized Allocation Low No and an Stream Substance Purity High The source was identified as New England Nuclear. Ormain 2: Test Design Metric 6: Randomized Allocation Low No regative control group was reported. Metric 6: Randomized Allocation Consistency of Exposure High A single dose was administened to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Administration Metric 6: Ressurement of Test Substance <th>Media, Path:</th> <th></th> <th></th> <th></th> <th>······································</th>	Media, Path:				······································	
Health Outcome: ADME (biotransformation) Chemical: Denmini Diversity phyladiae (DEHP) HERO D: 59542 Domain Of Metric ODEHP) Metric 1: Test Substance (dentity Medium The chemical was identified by name; (14C)-di-2-ethylhexylphhalate (DEHP). No CASRN or structure were provided. Metric 2: Test Substance Source High The source was identified as New England Nuclear. Metric 3: Test Substance Pource High The source was identified as New England Nuclear. Metric 4: Negative Controls VIA No negative control group was reported. Metric 6: Randomized Allocation Low No negative control group was reported. Metric 6: Randomized Allocation Low No negative control group was reported. Metric 6: Randomized Allocation Low No negative control group was reported. Metric 6: Randomized Allocation Low No negative control group was reported. Metric 6: Randomized Allocation Low No nandom allocation was reported. Metric 6: Randomized Allocation Low No nandom allocation was reported. Metric 6: Randomized Allocation Low No nandom allocation was reported. Metric 6: Consistency of Exposure Metric 6: Consistency of Exposure High Asingle does was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Delift was disolved in accentor introl does use and the system. Metric 10: Exposure Datation and Frequency High The 27-day reposure was sufficient. Metric 10: Exposure Datation and Frequency High The 27-day reposure was sufficient. Metric 11: Number of Exposure Groups/ N/A One exposure concentration was sufficient. Metric 12: Test of allow Solubility Limit Medium DEHP was disolved in accent and phylatic language of organisms were collected in the field. Age and sex were not provided in the system. Metric 13: Test Organism Characteristics Low The study did not report acclimation or pretreatment. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Metric 15: Number of Organisms and Low No replicates were reported. Metric 16: Significator Organisms and Low No repl	Taxa, Species, Age: I	Invertebrate;	Mollusks; Planorbis corneus; Not Applicab	le (e.g., fungi or algae st	udies) or Not Reported	
Chemical: Di-ethylhexyl phthalate (DEHP) HRO ID: 59542 Domain Metric Rating Comments Omain 1: Test Substance Metric 1: Metric 2: Test Substance Source Metric 3: Test Substance Purity Metric 4: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation Metric 6: Randomized Allocation Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Metric 8: Construction Metric 9: Measurement of Test Substance Metric 10: Exposure Characterization Metric 10: Exposure Characterization Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Metric 12: Testing at or Below Solution and Frequency Metric 11: Number of Exposure Oragin Metric 12: Testing at or Below Solution and Frequency Metric 12: Testing at or Below Solution and Frequency Metric 12: Testing at or Below Soluting aton Bulance was acterenther to adding it th	Health Outcome:	ADME (biotransformation)				
Disk Of Dis 59542 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Medium The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided. Metric 2: Test Substance Source High The source was identified as New England Nuclear. Metric 3: Test Substance Purity High The purity was 99-5%. Domain 2: Test Design Metric 6: Randomized Allocation Low Metric 5: Negative Controls Uninformative No random allocation was reported. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Low Metric 6: Consistency of Exposure Administration High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Administration Metric 9: Consentency of Exposure Concentration High A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. Metric 10: Exposure Duration and Frequency Metric 10: Exposure Duration and Frequency High The test substance was measured by TLC and liquid scinilitation. Final concentrations or sedware completed at the of of study.Massubalances were calculate	Chemical: I	Di-ethylhexy	l phthalate (DEHP)			
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Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Metric 12: Testing at or Below Solubility Limit Domain 4: Test Organism Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Number of Organisms and Metric 15: Outcome Assessment			Concentration		of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of study. Mass belances were calculated. The mass	
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Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Number of Organisms and Low No replicates were reported. Domain 5: Outcome Assessment Domain 5: Outcome Assessment Domain 5: Outcome Assessment	Ν	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solu-	
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Metric 15: Number of Organisms and Low No replicates were reported. Pomain 5: Outcome Assessment					bility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.	
Metric 13: Test Organism Characteristics Low Organisms were collected in the field. Age and sex were not provided in the study. Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Metric 15: Number of Organisms and Low No replicates were reported. Pomain 5: Outcome Assessment	Domain 4. Test Organism					
Metric 14: Acclimatization and Pretreatment Low The study did not report acclimation or pretreatment. Conditions Metric 15: Number of Organisms and Low No replicates were reported. Replicates per Group	Domain 1. Test Organism	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.	
Conditions Metric 15: Number of Organisms and Low No replicates were reported. Replicates per Group	l	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.	
Metric 15: Number of Organisms and Low No replicates were reported. Replicates per Group			Conditions		- · ·	
Comain 5: Outcome Assessment	Ν	Metric 15:	Number of Organisms and	Low	No replicates were reported.	
Jomain 5: Outcome Assessment			Replicates per Group			
	Domain 5: Outcome Asses	ssment				

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation:	Sodergren, A Environment	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 day	ys				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Mollusks; Planorbis corneus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	transformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	59542						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.			
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in the tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).			
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for the measurement of 14C DEHP.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP uptake by each organism.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal at- trition or health outcomes unrelated to the exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.			
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.			
Additional Comments:	This study of mesocosm. (may not be a DEHP + me	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites, the metabolites being phthalic acid and phthalic anhydride.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.				
Duration:	Environmen Overall Dura	tal Pollution 27(4):263-274. ation: > 21 days; Exposure Duration: > 21 d	ays		
Exposure Route, Media Pathy	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chemical of in	nterest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; ADME (biot Di-ethylhexy 59542	Arthropods; <i>Sialis sp.</i> ; Not Applicable (e.g., ransformation) /l phthalate (DEHP)	fungi or algae studies) o	or Not Reported	
Domain		Metric	Rating	Comments	
Domain 1: Test Substance	e				
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.	
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.	
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.	
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.	
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.	
Domain 3: Exposure Cha	racterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.	
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.	
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).	
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One exposure concentration was utilized in this study.	
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.	
Domain 4: Test Organism	1				
	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.	
	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.	
		Replicates per Group			
Domain 5: Outcome Asse	essment				

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		cor	ntinued from previou	s page		
Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 da	ys			
Exposure Route, Media, Path:	Aquatic (fre	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate	; Arthropods; Sialis sp.; Not Applicable (e.g., 1	fungi or algae studies)	or Not Reported		
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	59542					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.		
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).		
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP		
		Design and Procedures		uptake by each organism.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal a trition or health outcomes unrelated to the exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	lysis				
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.		
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.		
Additional Comments:	This study of mesocosm. may not be DEHP + me	collected 13 organisms (fish, invertebrates, a One tank containing the organisms was compleated acutely harmful to fish." The chosen concentre tabolites, the metabolites being phthalic acid a	nd plants) from unpo eted. Fish were separa ration was known to b and phthalic anhydride	olluted streams, as well as the collection of water and sediment for the ted in the tank. Toxicity was addressed as "the results indicate that DEHP be below that of which would cause acute toxicity. The authors measured		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.						
	Environmen	Environmental Pollution 27(4):263-274.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	lays				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	T (1 (weeks weeks weeks a state of the second state					
Taxa, Species, Age:	Invertebrate;	ADME (biotransformation)					
Chamical	ADME (010)	uransiormation)					
Unemical:	50542	yi phinalate (DEHP)					
neko id:	39342						
Domain		Metric	Rating	Comments			
Domain 1: Test Substanc	e						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.			
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.			
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.			
Domain 2: Test Design							
e	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.			
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.			
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.			
Domain 3: Exposure Cha	racterization						
	Metric 7:	Experimental System/Test Media	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.			
		Preparation					
	Metric 8:	Consistency of Exposure	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP.			
	M	Administration	TT' 1	DEHP was dissolved in acetone prior to adding it to the system.			
	Metric 9:	Measurement of Test Substance	High	The test substance was measured by TLC and liquid scintillation. Final concentrations			
		Concentration		isms were completed at the end of study. Mass balances were calculated. The mass			
				balance was DEHP + metabolites (phthalic acid and phthalic anhydride).			
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.			
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.			
Domain 4: Test Organisn	n		_				
	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.			
	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.			
		Replicates per Group	2011	no replicates noto reported.			
Domain 5: Outcome Ass	essment						

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page		
Study Citation:	Sodergren, A Environmen	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 day	ys			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study auth	ors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	-					
Taxa, Species, Age:	Invertebrate;	Invertebrate; Worms (e.g., Annelids, Nematodes); Tubijex sp.; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	ADME (biot	ADME (biotransformation)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	59542					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.		
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).		
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 14C DEHP.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into same experimental tank could affect 14C DEHP uptake by each organism.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal at- trition or health outcomes unrelated to the exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.		
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.		
Additional Comments:	This study of mesocosm. (may not be a DEHP + me	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites, the metabolites being phthalic acid and phthalic anhydride.				

Overall Quality Determination

Uninformative

Study Citation:	Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in Chlorella vulgaris.						
Duration: Exposure Route, Modio, Path:	Overall Dura Aquatic (fre	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; 1 ADME (bio Di-ethylhex) 679344	Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not transformation) yl phthalate (DEHP)	t Applicable (e.g., fungi or	r algae studies) or Not Reported			
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	High	Chemical identified by name			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory			
	Metric 3:	Test Substance Purity	High	purity reported as 99%			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	Uninformative	Study authors reported using an analytical control (no algae) and what appears to be a baseline control at time 0 hour. Did not use a negative control without test substance.			
	Metric 5:	Negative Control Response	Medium	Baseline control response noted on graphs and in equations			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations			
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistently administered to test organisms.			
	Metric 9:	Measurement of Test Substance	Medium	Concentrations were measured but only reported as log values on a graph			
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency differed significantly from typical study designs, concentrations never reached steady state			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration used			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized			
	Metric 15:	Conditions Number of Organisms and	Low	The number of test replicates was not reported			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Minor uncertainties or limitations were identified regarding organism environmental conditions as nutrient ratios were altered for each test			
		С	Continued on next page	••			

Diethylhexyl Phthalate

		con	tinued from previou	s page		
Study Citation:	Chi, J., Li, B Journal of Ei	Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in Chlorella vulgaris. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 42(2):179-183.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10 d	lays			
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Chlorella vulgaris; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	679344					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes but the difference between biodegradation and bioconcentration wasn't clear		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups		
Domain 6: Confounding	g / Variable Cor	ntrol				
· · · · · · · · · · · · · · · · · · ·	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	only one concentration used		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment over time		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained		
Additional Comments:	DEHP expos	EHP exposure at various N/P levels.				

Overall Quality Determination

Uninformative

Study Citation:	Shen, C., Wang, Y., Shen, Q., i, Wang, L., i, Lu, Y., Li, X., in, Wei, J., ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga Chlorella vulgaris. IOP Conference Series: Earth and Environmental Science 227(5):052054.						
Duration: Exposure Route, Media, Path:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Chlorella vulgaris; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Oxidative	stress (includin	ng redox biology)			
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5692135						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by name and CAS no.			
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure), but it was unclear whether it was a water or solvent control.			
	Metric 5:	Negative Control Response	High	The biological response of the control group was adequate.			
	Metric 6:	Randomized Allocation	Low	This is an algal study and reporting of random allocations is limited.			
Domain 3: Exposure Cha	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance during exposure. Concentrations of the test substance were not measured during the study.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type and outcomes of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for the purpose of the study.			
	Metric 12:	Testing at or Below Solubility Limit	Low	A solvent was used to prepare test solutions, but it was unclear whether the control had a solvent or not. The control response was adequate. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).			
Domain 4: Test Organisi	m Metric 13:	Test Organism Characteristics	High	The source was reported, and the test organisms were appropriate for evaluation of the specific outcome of interest.			
		Contin	ued on next pa	nge			

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Diethylhexyl Phthalate

		conti	nued from previ	ous page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Shen, C., Wa damage in th Overall Dura Aquatic (fre uptake route Vegetation; 1 Mechanistic Di-ethylhexy	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga Chlorella vulgaris. IOP Conference Series: Earth and Environmental Science 227(5):052054. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology) Di-ethylbexyl phthalate (DEHP)					
HERO ID:	5692135						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether the pretreatment conditions were the same for control			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The test vessels were inoculated at an initial concentration of 1.2×10 ⁶ cells/mL. Each test concentration had four replicates.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate and whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies for hydrogen peroxide and malondialdehyde content, and superoxide dismutase and glutathione peroxidase activities were reported.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported. It was not reported whether the biomarker measurements were done at the end of the growth experiment.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal Metric 21:	ysis Statistical Methods	Low	In the methods section, it was reported that Student's t test was conducted, but this is not an appropriate test when there are more than 2 treatment groups. ANOVA is the appropriate statistical test to compare more than 2 groups and helps to control Type 1			
				error.			

Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes. Additional Comments: It was unclear whether a water control or a solvent control was used. Incorrect statistical analysis was conducted, and data enabling an independent statistical analysis were not provided. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed

High

Data for exposure-related findings were presented for each treatment and control group.

in the final scope (0.27 mg/L).

Reporting of Data

Metric 22:

Overall Quality Determination

Medium

Study Citation:	Shen, C., Wang, Y., Shen, Q., i, Wang, L., i, Lu, Y., Li, X., in, Wei, J., ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative
	damage in the microalga Chlorella vulgaris. IOP Conference Series: Earth and Environmental Science 227(5):052054.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route,	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact
Media, Path:	uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Chlorella vulgaris; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	5692135

Domain		Metric	Rating	Comments		
Domain 1: Test Substance	e					
	Metric 1:	Test Substance Identity	High	The test substance was identified by name and CAS no.		
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure), but it was unclear whether it was a water or solvent control.		
	Metric 5:	Negative Control Response	High	The biological response of the control group was adequate.		
	Metric 6:	Randomized Allocation	Low	This is an algal study and reporting of random allocations is limited.		
Domain 3: Exposure Cha	aracterization		_			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance during exposure. Concen- trations of the test substance were not measured during the study.		
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure (5 days) was reported but was higher than the duration recom- mended by OECD 201 test guidelines (72 hours), and no justification was provided.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for the purpose of the study.		
	Metric 12:	Testing at or Below Solubility Limit	Low	A solvent was used to prepare test solutions, but it was unclear whether the control had a solvent or not. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).		
Domain 4: Test Organism	n					
6	Metric 13:	Test Organism Characteristics	High	The source was reported, and the test organisms were appropriate for evaluation of the specific outcome of interest.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether the pretreatment conditions were the same for the control and the exposed groups.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The test vessels were inoculated at an initial concentration of 1.2×10 ⁶ cells/mL. Each test concentration had four replicates.		
Continued on next page						

Diethylhexyl Phthalate

		conti	nued from p	revious page				
Study Citation: Duration:	Shen, C., Wa damage in th Overall Dura	Shen, C., Wang, Y., Shen, Q., i, Wang, L., i, Lu, Y., Li, X., in, Wei, J., ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga Chlorella vulgaris. IOP Conference Series: Earth and Environmental Science 227(5):052054. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days						
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact						
Media, Path:	uptake route	uptake route)						
Taxa, Species, Age:	Vegetation;	Non-vascular Plants; <i>Chlorella vulgaris</i> ; No	of Applicable	e (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	lt/Growth						
UEDO ID.	Di-ethylnexy	yi phthalate (DEHP)						
HERO ID:	3092133							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate and whether differences occurred between control and exposed populations.				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was stated the algal cell density of the algal culture was determined using a Neubauer haemocytometer, but the methods were not provided.				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.				
Domain 7: Data Present	tation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted for the growth experiment data, and data enabling an independent statistical analysis were not provided. A 96h EC 50 value was reported, but the statistical method used was not reported. Mean algal density data was given in the figure without any measure of variability. In the methods section, it was reported that Student's t test test was conducted, but this is not an appropriate test when there are more than 2 treatment groups.				
	Metric 22:	Reporting of Data	Low	Cell density data was presented without measures of variability. Specific growth rate was calculated but not reported. The 96H EC value was reported without confidence intervals.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals), and insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.				
Additional Comments:	It was unclea an independent solubility lis	ar whether a water control or a solvent con ent statistical analysis were not provided. T ted in the final scope (0.27 mg/L).	ntrol was use The solubility	d. Statistical analysis was not conducted for the growth experiment and data enabling / limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the				

Overall Quality Determination

Low

Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology						
Duration	and Chemist	try 22(12):3037-3043. ation: 0 - 4 days (0-96h): Exposure Duration	1 - 4 days (0.96h)				
Exposure Route.	Aquatic (fre	shwater): Water: Not determined by study a	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Media, Path:	i iquaite (iite		anois (nei, enemear or m				
Taxa, Species, Age:	Vegetation;	Vegetation; Non-vascular Plants; Pseudokirchneriella subcapitata; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhex	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	789536						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively. Nomenclature, CASRN, and structure were reported.			
	Metric 2:	Test Substance Source	High	DEHP was purchased from Merck Eurolab (Stockholm, Sweden), but the test substance identity was NOT analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Percent purity was reported as >98%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the control group was reported. The control group had a growth rate 1.6-1.8/ d during the 72 hour of incubation.			
	Metric 6:	Randomized Allocation	Low	There were minor limitations in the allocation method that are unlikely to have a sub- stantial impact on results. This was an algal study, and reporting of random allocations are limited.			
Domain 3: Exposure Ch	naracterization						
2 onian of 2nposare of	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the			
	Matria Q.	Consistences of Franceson	TT: -1-	exposure.			
	Metric 8:	Administration	підп	consistently across study groups			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were measured but not reported.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type. The algal growth inhibition test was conducted for 72 hours			
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the exposure concentrations or on the spacing of expo-			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	sure levels. Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit.			
Domain 4: Test Organis	m						
_ shan in rost organis	Metric 13:	Test Organism Characteristics	Low	The source of algae was not reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
			Continued on next nage				
			second on next page .				

Diethylhexyl Phthalate

HERO ID: 789536 Table: 1 of 1

		con	tinued from previou	s page				
Study Citation:	Jonsson, S.,	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology						
Duration:	Overall Dur	ation: 0 - 4 days (0-96h): Exposure Duration: 0) - 4 days (0-96h)					
Exposure Route.	Aquatic (fre	Aquatic (freshwater). Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media. Path:	i iquano (ire							
Taxa, Species, Age:	Vegetation:	Non-vascular Plants: Pseudokirchneriella subc	<i>apitata</i> : Not Applicab	le (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmer	nt/Growth						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	789536	, , , , , , , , , , , , , , , , , , ,						
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test vessels were inoculated to achieve a cell density of 10 ⁴ cells/ml. Replicates were not reported.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were conducive to the maintenance of health. Typical control growth rates of 1.6-1.8/d were observed in controls.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology was reported. The cited reference (Mayer et al. 1997) has the detailed methodology.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups. Samples were taken from each test flask and the controls every 24 h to determine growth rates.				
Domain 6: Confounding	y / Variable Co	ntrol						
Doman of Company	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
Domain 7: Data Present	tation and Anal	lycic						
	Metric 21:	Statistical Methods	High	Nonlinear regression analysis was conducted to estimate EC values and confidence intervals.				
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. Only the EC 10 and EC 50 values were provided.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	There were no unexpected outcomes.				
Additional Comments:	The exposur data were no	re concentrations, spacing of exposure levels, and ot provided for each of the treatment groups and	nd control response w d control. Only EC 10	ere not reported. Measured concentrations were not reported. Growth rate and EC 50 values were reported.				

Overall Quality Determination

Uninformative

Study Citation:	Bionomics,,	Bionomics, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga Selenastrum capricornutum.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days				
Exposure Route,	Aquatic (fre	shwater); Cell Culture Media; Not determ	ined by stud	y authors (i.e., chemical of interest in exposure water, but unable to determine exact			
Media, Path:	uptake route)					
Taxa, Species, Age:	Vegetation; 1	Vegetation; Non-vascular Plants; Selenastrium capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1316196						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the chemical was not included in the technical report.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade were not included in the technical report.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Negative controls were included in the study.			
	Metric 5:	Negative Control Response	High	There was an adequate response from the negative controls.			
	Metric 6:	Randomized Allocation	Medium	The allocation of algae for the experiments was not described in the study.			
Domain 3: Exposure Ch	aracterization						
· · · · · · ·	Metric 7:	Experimental System/Test Media	High	The study followed OECD 201 (1981) guidelines and included any deviations.			
		Preparation	6				
	Metric 8:	Consistency of Exposure	High	The exposure administration was reported as consistent.			
		Administration	U				
	Metric 9:	Measurement of Test Substance	High	Appendix A includes the analytical measurement of the chemical, gas-liquid chromatog-			
		Concentration		raphy with electron capture detection. Concentrations were measured throughout the 10			
				days. The initial and final concentrations can be found in the text.			
	Metric 10:	Exposure Duration and Frequency	High	The 10-day exposure was adequate for the study.			
	Metric 11:	Number of Exposure Groups/	Medium	There was an appropriate number of exposure groups.			
	M-4.:- 10.	Spacing of Exposure Levels	TT: -1-				
	Metric 12:	Testing at or Below Solubility Limit	High	No response was observed for the single concentration recorded below the solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source and the details of the algae were limited.			
	Metric 14:	Acclimatization and Pretreatment	Medium	Acclimatization details were limited.			
		Conditions					
	Metric 15:	Number of Organisms and	Medium	The study followed OECD 201 (1981) guidelines using adequate numbers of organisms.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome for the chemical was described in detail in results.			
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Continued on next page							

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1316196 Table: 1 of 1

		conti	nued from p	revious page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga Selenastrum capricornutum. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Non-vascular Plants; <i>Selenastrium capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP) 1316196			
Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were reported consistently.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences were reported among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistics used include, moving average angle analysis, probit analysis, and binomial probability.
	Metric 22:	Reporting of Data	Medium	Data was reported for growth.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	Initial studies to determine test concentrations concluded that no response from the algae was observed at concentrations below the water solubility limit, therefore 1 concentration (below the solubility limit) was reported with no response.			
Overall Quali	ty Detern	nination	High	

Study Citation: Duration: Exposure Route, Media, Path:	Adams, W. J organisms. E Overall Dura Aquatic (fres	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vegetation; 1	Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.			
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.			
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A negative control was reported.			
	Metric 5:	Negative Control Response	High	The control response was acceptable.			
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures taken to prevent volatilization were not reported.			
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.			
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test.			
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.			
Domain 4. Test Organia	~						
Domain 4: Test Organisi	II Metric 13:	Test Organism Characteristics	Low	The source was not reported			
	Metric 13.	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported			
		Conditions	man	in appropriate accontation period for the test was reported.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.			
Domain 5: Outcome Age	assmant						

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

HERO ID: 1321996 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Adams, W. J	I., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic			
	organisms. I	Environmental Toxicology and Chemistry 1	4(9):1569-1:	574.			
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Non-vascular Plants; Selenastrum capricor	<i>nutum</i> ; Not A	Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	tt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.			
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.			
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent across groups.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.			
		Design and Procedures	6	e I			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.			
Domain 7: Data Present	ation and Anal	veie					
Domain 7. Duta 11050m	Metric 21	Statistical Methods	High	Statistical methods were performed and described			
	Metric 22	Reporting of Data	Medium	Only treatment endpoints were reported			
	Metric 22.	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported			
	metric 23.	Explanation of Onexpected Outcomes	Ingn	no unexpected outcomes were reported.			
Additional Comments:	None						
<u> </u>		•	TT -				
Overall Quali	ty Deterr	nination	High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.						
Duration:	Environmen Overall Dura	tal Pollution 27(4):263-274. ation: > 21 days: Exposure Duration: > 21 d	avs					
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Chara chara</i> ; Not Applicable	e (e.g., fungi or algae stu	dies) or Not Reported				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	59542							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		M F					
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.				
	Metric 2:	Test Substance Source	High	The source was identified as New England Nuclear.				
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.				
Domain 2: Test Design								
C C	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.				
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.				
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.				
Domain 3: Exposure Ch	aracterization							
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.				
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.				
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organ- isms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).				
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.				
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L, which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.				
Domain 4: Test Organis	m							
Domain 1. 10st Organis	Metric 13:	Test Organism Characteristics	Low	Organisms were collected in the field. Age and sex were not provided in the study				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report acclimation or pretreatment.				
	Metric 15:	Conditions Number of Organisms and	Low	No replicates were reported.				
		Replicates per Group		- •				
Domain 5: Outcome As	sessment							

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

Study Citation:	Sodergren, A	odergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.					
·	Environmen	Environmental Pollution 27(4):263-274.					
Duration:	Overall Dura	Diverall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vegetation; Vascular Plants; Chara chara; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biotransformation)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	59542						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.			
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).			
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion o the study (27 days). Water samples were obtained every 5 days for measurement of 14 DEHP.			
Domain 6: Confounding	g / Variable Co	ntrol					
c	Metric 19:	Confounding Variables in Test	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP			
		Design and Procedures		uptake by each organism.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.			
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.			
Additional Comments:	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites the metabolites being phthalic acid and phthalic anydride.						

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Study Citation:	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study	authors (i.e., chemical of inter	est in exposure water, but unable to determine exact uptake route)			
Media, Path:	Vagatation	Vacaular Dianta, Laura win an Nat Appli	ashla (a.a. funci an alaga studi	an Nat Deposited			
Taxa, Species, Age:	Machanistia	Piemerkers (appears and affect) Oridat	two stress (including redex high	legy) Distographics			
Chemical:	Di_ethylbeyy	Di-ethylbexyl phthalate (DFHP)					
HERO ID:	1340050						
Domain	15 10050	Metric	Dating	Comments			
Domain 1: Test Substan	ice	Mette	Katilig	Comments			
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	The chemical was identified by name			
	Metric 2:	Test Substance Source	Low	The source was not reported			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported			
	Weute 5.	Test Substance Funty	Low	Turty and/or grade of the est substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
		Concentration		1			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a			
		Spacing of Exposure Levels		dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
<i>.</i>	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15.	Conditions Number of Organisms and	Low	The number of test organisms was not reported			
	metho 15.	Replicates per Group	2011	The number of test organisms was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Minor uncertainties were identified regarding environmental conditions, but these are not likely to have a substantial impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
			Continued on next page				

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Diethylhexyl Phthalate

HERO ID: 1340050 Table: 1 of 1

		co	ntinued from previous	page			
Study Citation: Duration:	Xu, G., Wu, Overall Dura	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; Mechanistic	Vegetation; Vascular Plants; <i>Lemna minor</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis					
HERO ID:	1340050	1340050					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups in test organism attrition or outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Uninformative	Although it appears there are error bars in the figures, statistical analysis was not con- ducted nor described. The study authors reported conducting the experiment three times, but it is unclear if individual test concentrations were conducted three times or if the entire experiment was conducted three times (nine different replicates of each test con- centrations or three replicates total).			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	Low High	Continuous data were presented without measures of sample size of each group. There were no unexpected outcomes.			
Additional Comments:	This is a me	chanistic study.					
Overall Quali	ty Deterr	nination	Uninformative	9			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem.						
Duration	Environmen Overall Dura	vironmental Pollution 27(4):263-274. erall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study au	thors (i.e., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Media, Path:	-						
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; <i>Mentha aquatica</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (bioi	ADME (biotransformation)					
HERO ID:	59542						
Domain		Metric Rating Comments					
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name: (14C)-di-2-ethylhexylphthalate (DEHP). No CASRN or structure were provided.			
	Metric 2:	Test Substance Source	High	the source was identified as New England Nuclear.			
	Metric 3:	Test Substance Purity	High	The purity was 99.5%.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	Uninformative	No negative control group was reported.			
	Metric 5:	Negative Control Response	N/A	No negative control group was reported.			
	Metric 6:	Randomized Allocation	Low	No random allocation was reported.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media Preparation	Medium	Biomass loading and placement of organisms in the 20L tank were not reported.			
	Metric 8:	Consistency of Exposure Administration	High	A single dose was administered to the water in a 20-liter tank at 1.43 mg/L 14C DEHP. DEHP was dissolved in acetone prior to adding it to the system.			
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured by TLC and liquid scintillation. Final concentrations of sediment, glass walls, surface microlayer, suspended material, and groups of organisms were completed at the end of the study. Mass balances were calculated. The mass balance was DEHP + metabolites (phthalic acid and phthalic anhydride).			
	Metric 10:	Exposure Duration and Frequency	High	The 27-day exposure was sufficient.			
	Metric 11:	Number of Exposure Groups/	N/A	One exposure concentration was utilized in this study.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	DEHP was dissolved in water at 1.43 mg/L which is greater than the approximate solubility in water (0.3 mg/L). Use of acetone as a solvent may increase solubility slightly.			
Demain 4: Test Onessie							
Domain 4: Test Organis	Metric 13.	Test Organism Characteristics	Low	Organisms were collected in the field. Are and say were not provided in the study			
	Metric 14	Acclimatization and Pretreatment	LOW	The study did not report acclimation or pretreatment			
	mente 14.	Conditions	LOW	The study did not report accumution of protoaution.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replicates were reported.			

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 59542 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation:	Sodergren, A Environment	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Vegetation; Vascular Plants; Mentha aquatica; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biotransformation)						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	59542						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Biomass loading was not described in adequate detail. Details of the feed for fish were not well described.			
	Metric 17:	Outcome Assessment Methodology	Medium	Extraction and measurement of 14C DEHP in tissue of organisms as well as water and sediment were described, but details of organism preparation for extraction were not described (euthanasia, if applicable, or harvesting of plant material).			
	Metric 18:	Consistency of Outcome Assessment	High	Extraction of organisms for measurement of 14C DEHP took place at the conclusion of the study (27 days). Water samples were obtained every 5 days for measurement of 140 DEHP.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Multiple organisms loaded into the same experimental tank could affect 14C DEHP uptake by each organism.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistics were not possible with one study group.			
	Metric 22:	Reporting of Data	High	14C DEHP concentrations and BCF values were shown in Tables 1 and 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variability was reported.			
Additional Comments:	This study collected 13 organisms (fish, invertebrates, and plants) from unpolluted streams, as well as the collection of water and sediment for the mesocosm. One tank containing the organisms was completed. Fish were separated in the tank. Toxicity was addressed as "the results indicate that DEHP may not be acutely harmful to fish." The chosen concentration was known to be below that of which would cause acute toxicity. The authors measured DEHP + metabolites the metabolites being phthalic acid and phthalic anbydride.						

Overall Quality Determination

Uninformative

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Study Citation:	Xu, G., Wu, Overall Dura	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981. Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days					
Exposure Route.	Aquatic (free	shwater): Water: Not determined by study	v authors (i.e., chemical of inter	est in exposure water, but unable to determine exact uptake route)			
Media. Path:	riquite (free	invalor), valor, rot actornined by stady	autions (nei, enemieur of mier				
Taxa, Species, Age:	Vegetation: V	Vascular Plants: <i>Spirodela polvrhiza</i> : Not	Applicable (e.g., fungi or algae	e studies) or Not Reported			
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Oxidat	tive stress (including redox biol	ogy)-Photosynthesis			
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	1340050	340050					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	the chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions were unlikely to have a substantial impact on results.			
	M	Administration	T				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a			
		Spacing of Exposure Levels	8	dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4: Test Organis	m						
6	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Minor uncertainties were identified regarding environmental conditions, but these are not likely to have a substantial impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Continued on next page						

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Diethylhexyl Phthalate

		co	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media Path:	Xu, G., Wu, Overall Dura Aquatic (fre	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Mechanistic Di-ethylhex 1340050	Vegetation; Vascular Plants; <i>Spirodela polyrhiza</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis Di-ethylhexyl phthalate (DEHP) 1340050				
Domain		Comments				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups in test organism attrition or outcomes unrelated to the exposure.		
Domain 7: Data Present	tation and Anal	lysis				
	Metric 21:	Statistical Methods	Uninformative	Although it appears there are error bars in the figures, statistical analysis was not con- ducted nor described. The study authors reported conducting the experiment three times, buy it is unclear if individual test concentrations were conducted three times or if the entire experiment was conducted three times (nine different replicates of each test con- centrations or three replicates total).		
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of sample size of each group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This is a me	chanistic study.				
Overall Quali	ty Deterr	nination	Uninformative	e		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Gao, M., Do (2-ethylhexy Overall Dura Aquatic (fres Vegetation; V Reproductive Di-ethylhexy 3515118	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 3515118				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS.		
	Metric 2:	Test Substance Source	Low	The source was reported as J&K Scientific Ltd. (Beijing, China), but the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 96.8%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were adequately reported. Petri dishes were covered with a lid to prevent evaporation. Water loss was checked every 24 hours, but loss of test substance was not measured.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Matria 10.	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported and eppropriate for the study type		
	Metric 11.	Number of Exposure Groups/	High	The number of exposure aroung and the spacing of exposure levels were evitable for a		
	wiente 11.	Spacing of Exposure Levels	Ingn	dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the bio- logical response of the solvent control was acceptable, and no interactions are expected between the solvent and test substance.		
Domain 4. Test Orregies	~					
Domain 4: Test Organisi	II Matric 12:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source		
	Metric 14	A colimatization and Protreatment	High	All pretreatment conditions were the same for control and avnosed organisms		
	wieure 14:		rigi	An pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
		Replicates per Group				

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

		cont	inued from p	revious page			
Study Citation:	Gao, M., Do (2-ethylhexy	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	-						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Triticum sp.; Jinnong 7; N	lot Applicable	e (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3515118						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health. The germination experiment was conducted in "a growth chamber in total darkness at temperature of 25 ± 1 oC and humidity of 80%."			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (germination rate, and root and shoot length) reported the intended outcome of interest but was not described adequately.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding / Variable Control							
·	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			

Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	Statistical methods were described well.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, for germination rate (Fig 1) and root/shoot elongation (Fig 2). IC 10 and IC50 values were provided for root and shoot elongation.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	s: This evaluation form is relevant for germination rate and shoot/root growth.					

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation: Vascular Plants: <i>Triticum sp</i> : Jinnong 7: Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome: Chemical: HFRO ID:	Development Di-ethylhexy	Development/Growth Di-ethylhexyl phthalate (DEHP)					
Domain	5515110	Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS.			
	Metric 2:	Test Substance Source	Low	The source was reported as J&K Scientific Ltd. (Beijing, China), but the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 96.8%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the test media preparation methods were adequately re- ported. The test solutions were replenished daily.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the bio- logical response of the solvent control was acceptable.			
Domain 4: Test Organisr	n						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome Ass	sessment						
		Conti	inued on nex	t page			

Diethylhexyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route.	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquatic (freehwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route).					
Media. Path:	riquite (iie	situati, traci, iter determined by study t	autions (i.e.,			
Taxa, Species, Age:	Vegetation; Vascular Plants; Triticum sp.; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	3515118					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health. "The experiments were performed in an artificial climate chamber with alternating temperatures of 25 ± 1 oC (12 h light) and 20 ± 1 oC (12 h dark), 60% rela- tive humidity, and a light intensity of 40 uM/ms".		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest. Roots were scanned using an EPSON Expression, and measurements (total root length, total root surface area, average root diameter, and the number of root tips and hairs) were obtained from digital images.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	y / Variable Co	ntrol				
Domain of Companying	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures	e			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	veis				
Domain 7. Data i leselit	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 2 and Fig 3).		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Root morph	ology measurements included total root le	ength, total r	oot surface area, average root diameter, and the number of root tips and hairs. The		

Overall Quality Determination

exposure concentrations were not verified.

High
Domain 5: Outcome Assessment

Metric 16:

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 3515118 Table: 2 of 3

Stardar Citations	C. M. D	and V. Zhang, Z. Cana, W. O', V. (20	17) Creat (1							
Study Citation:	Gao, M., D	ong, Y., Zhang, Z., Song, W., Qi, Y. (20	1/). Growth	and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di						
Duration	Overall Dur	(1) phinalale stress. Chemosphere 172(Else	evier):418-420	5.						
Exposure Route	Aquatic (fre	shwater): Water: Not determined by study	authors (i e	chemical of interest in exposure water, but unable to determine exact untake route)						
Media, Path:	riquitie (iie	Vegetation: Vascular Plants: Triticum sp.: Jinnong 7: Not Applicable (e.g., fungi or algae studies) or Not Reported								
Taxa, Species, Age:	Vegetation:									
Health Outcome:	ADME (bio	transformation)	tot rippileuoit	(e.g., fungi of algue studies) of five reported						
Chemical:	Di-ethylhex	vl phthalate (DEHP)								
HERO ID:	3515118	() p								
Domain		Metric	Rating	Comments						
Domain 1: Test Substan	ce		ituting							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS.						
	Metric 2:	Test Substance Source	Low	The source was reported as J&K Scientific Ltd. (Beijing, China), but the test substance						
				identity was not analytically verified by the performing laboratory.						
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 96.8%.						
Domain 2: Test Design	34.1.4									
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.						
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.						
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.						
Domain 3: Exposure Ch	aracterization									
- · · · · · · · · · · · · · · · · · · ·	Metric 7:	Experimental System/Test Media	Medium	The experimental system and the test media preparation methods were adequately re-						
		Preparation		ported. The test solutions were replenished daily.						
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.						
		Administration	C							
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.						
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.						
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a						
		Spacing of Exposure Levels	C	dose response.						
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration was reported but not the biological response of the solvent control.						
Domain 4: Test Organis	m		TT' 1							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.						
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.						
	Metric 15:	Number of Organisms and	Medium	The number of test organisms and replicates were reported and sufficient to characterize						

Number of Organisms and
Replicates per GroupMediumThe number of test organisms and replicates were reported and sufficient to characterize
toxicological effects.

Adequacy of Test ConditionsHighEnvironmental conditions of the test system were conducive to the maintenance of organism health. "The experiments were performed in an artificial climate chamber with alternating temperatures of $25 \pm 10C$ (12 h light) and $20 \pm 10C$ (12 h dark), 60% relative humidity, and a light intensity of 40 uM/ms".

Continued on next page ...

Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Gao, M., Do (2-ethylhexy	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:		• •					
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Triticum sp.; Jinnong 7; No	ot Applicable	e (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	3515118	- F					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (for determining DBP and DEHP in shoot and root) reported the intended outcome of interest. Key details for HLPC analysis (method detection limit, percent recovery, etc.) were not provided.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment group. The control			
				group response was not reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	The control	group was not analyzed for DEHP. The exp	osure concer	ntrations were not verified.			
Overall Quali	ty Detern	nination	High				

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HERO ID: 3515118 Table: 3 of 3

Study Citation: Duration: Exposure Route,	Gao, M., Do (2-ethylhexy Overall Dura Aquatic (fres	ong, Y., Zhang, Z., Song, W., Qi, Y. (20 I) phthalate stress. Chemosphere 172(Else tion: 11 - 21 days; Exposure Duration: 11 hwater); Water; Not determined by study	17). Growth evier):418-428 - 21 days authors (i.e.,	and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di 3. chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; V Mechanistic- Di-ethylhexy	egetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported lechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology) i-ethylhexyl phthalate (DEHP)					
HERO ID:	3515118						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		TT' 1				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS.			
	Metric 2:	Test Substance Source	Low	The source was reported as J&K Scientific Ltd. (Beijing, China), but the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 96.8%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
	, . , .						
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the test media preparation methods were adequately re- ported. The test solutions were replenished daily.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the bio- logical response of the solvent control was acceptable, and no interactions are expected between the solvent and test substance.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome Ass	sessment						
		Cont	inued on nex	at page			

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 3515118 Table: 3 of 3

		conti	nued from p	previous page				
Study Citation:	Gao, M., Do	Gao, M., Dong, Y., Zhang, Z., Song, W., Qi, Y. (2017). Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di (2-ethylhexyl) phthalate stress. Chemosphere 172(Elsevier):418-428.						
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Faxa, Species, Age:	Vegetation; V	Vascular Plants; Triticum sp.; Jinnong 7; No	ot Applicable	e (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Oxidativ	ve stress (inc	luding redox biology)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	3515118							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health. "The experiments were performed in an artificial climate chamber with alternating temperatures of 25 ± 1 oC (12 h light) and 20 ± 1 oC (12 h dark), 60% rela- tive humidity, and a light intensity of 40 uM/ms".				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodologies for enzyme activities, lipid peroxidation and permeability of plasma membrane were not clearly reported.				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
Domain 6. Confounding	/ Variable Cou	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7. Data Present	ation and Anal	veic						
	Metric 21.	Statistical Methods	High	Statistical methods were adequately described				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group for all mechanistic end points (Figures 4, 5 and 6).				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	This evaluation membrane p	ion form is relevant to all mechanistic end ermeability.	lpoints inclu	ding antioxidant enzyme activities, lipid peroxidation, O2 accumulation, and plasn				

Study Citation:	Heitmuller, I of Environm	P. T., Hollister, T. A., Parrish, P. R. (1981). A ental Contamination and Toxicology 27(5):	Acute toxicity of 596-604.	54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus). Bulletin				
Exposure Route,	Aquatic (ma	Advatic (marine): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	1							
Taxa, Species, Age:	Vertebrate; H	Fish; Cyprinodon variegatus; Juvenile						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	18110	() - P						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		т					
	Metric 1: Metric 2:	Test Substance Identity	Low	The source of the DEHP was not reported				
	Metric 3:	Test Substance Purity	High	All the chemicals used in this study were reported to be analytical grade with a mini- mum purity of 80%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using negative controls that contained any of the solvents or carriers at the highest levels used in the test concentrations.				
	Metric 5:	Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Domain of Exposure of	Metric 7:	Experimental System/Test Media Preparation	High	Tests were reported to be static tests. The test solution was prepared by either adding the appropriate amount of weighed test substance to each test container, or by adding the appropriate volume of test substance and solvent/carrier to each test container.				
	Metric 8:	Consistency of Exposure Administration	Medium	All tests were for 96h. Study authors reported using two different sized test containers, a 4L container size and a 19L container size. This would most likely not have a substantial impact on the results.				
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if exposure concentrations were measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	This was a 96h acute toxicity test, which is typical for fish species.				
	Metric 11:	Number of Exposure Groups/	Low	The study authors did not report the number of exposure groups or the spacing of the				
		Spacing of Exposure Levels		exposure groups. Only LC50 values were reported. However, the cited methods (EPA 1975) specifies that "for determination of an LC50 or an EC50, a control and at least five concentrations of toxicant in a geometric series should be used."				
	Metric 12:	Testing at or Below Solubility Limit	Low	Study authors did not report the exposure concentrations used, so it is unable to be de- termined if the exposure values were below the solubility limit. It was reported that solvents/carriers were used when necessary.				
Domain 4: Test Organis	m							
-	Metric 13:	Test Organism Characteristics	High	Test organisms were hatched and reared at BMRL and were originally from EPA in Gulf Breeze, FL. Juvenile organisms were used for the study.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions in any way.				
		Conti	nued on next pa	nge				

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Heitmuller, I of Environm	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Cyprinodon variegatus; Juvenile					
Health Outcome:	Mortality	Iortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	18110						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test chamber. The number of replicates was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were fed until the start of the study. Before the start of the study, they were kept at 25-31 C in seawater with ambient salinity. The actual temperature during the study was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–LC50 values/mortality.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Organisms were checked for mortality every 24h for 96h.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated in any way.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequate and described in the text.			
	Metric 22:	Reporting of Data	Low	Only LC50 values were reported. There was no raw data reported for mortalities in test concentrations or controls.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.			
Additional Comments:	This portion selected as the table of	of the evaluation is on the effect of dietheth	ylhexyl phthala	te on juvenile sheepshead minnows. LC50 values were reported, so mortality was			

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path:	Ye, T., Kang dysfunction Overall Dura Aquatic (ma	, M., Huang, Q., Fang, C., Chen, Y., Shen, H and endocrine disruption in marine medaka ation: > 21 days; Exposure Duration: > 21 rine); Water; Not determined by study author	I., Dong, S. (2014 (Oryzias melast days prs (i.e., chemica	4). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive igma). Aquatic Toxicology 146:115-126.l of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias melastigma; Adult		
Health Outcome:	Developmen	t/Growth		
Chemical:	2510010	yi phthalate (DEHP)		
Domain	2515010	Metric	Rating	Comments
Domain 1: Test Substan	ce	Weute	Ruting	Comments
	Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the sub- stance identity was verified.
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water.
	Metric 5:	Negative Control Response	High	The control responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not state if the fish were allocated at random.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	DEHP was dissolved in dimethyl sulfoxide. The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure. Concentrations were not measured.
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to examine growth and reproductive effects (6 months).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1 mg/L of DEHP, 0.5 mg/L of DEHP.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected between the solvent and test substance).
Domain 4: Test Organis	m			
0	Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the ex- posure was as follows: "Embryos were collected from the abdomens of healthy females, and the larvae hatched within one week were used for the subsequent exposure experi- ment."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.
		Conti	nued on next pa	ge

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Ye, T., Kang dysfunction	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquatic Toxicology 146:115-126.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	days				
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study autho	rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	T 7 , 1 , T						
Taxa, Species, Age:	Vertebrate; F	rish; <i>Oryzias melastigma</i> ; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	(I phthalate (DEHP)					
HERO ID:	2519010						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	50 fish per replicate were used, and there were 3 replicates per treatment group.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Housing and environmental conditions were appropriate for the marine medaka. The fish were raised in artificial seawater under standard laboratory conditions of 28 ± 1 °C on a 14:10 light/dark photoperiod. The fish were fed with freshly hatched Artemia nauplii twice daily.			
	Metric 17:	Outcome Assessment Methodology	High	The morphometric endpoints of condition factor (K), brain and gonadal somatic indices (BSA, GSI) were sensitive and appropriate.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment methods were reported and were the same across groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
·	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Information that could be used to compare environmental conditions across groups (measured concentrations, monitoring data for pH, DO, etc.) was limited.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested differences occurred that were unrelated to exposure.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	The authors used one-way ANOVAs and Tukey's post hoc tests.			
	Metric 22:	Reporting of Data	High	Means and SEM for both treatments and the control were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors do not describe any unexpected outcomes.			
Additional Comments:	The study ex evaluation for exposure con	camines the effects of 6 months of exposure to form is relevant to the morphometric endpoin acentrations.	to DEHP, and ev ts: condition fac	valuates a number of apical and mechanistic endpoints in the marine medaka. This etor (K), brain and gonadal somatic indices (BSA, GSI). The study does not verify			

Overall Quality Determination

Study Citation:	4). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive								
	dysfunction	and endocrine disruption in marine medaka	(Oryzias melast	igma). Aquatic Toxicology 146:115-126.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days						
Exposure Route,	Aquatic (ma	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:									
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Oryzias melastigma; Adult							
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)							
Chemical:	Di-ethylhex	l phthalate (DEHP)							
HERO ID:	2519010								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.					
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the sub- stance identity was verified.					
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.					
Domain 2: Test Design	N		TT: 1						
	Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water.					
	Metric 5:	Negative Control Response	High	The control responses were adequate.					
	Metric 6:	Randomized Allocation	Low	Researchers did not state if the fish were allocated at random.					
Domain 3: Exposure Ch	aracterization								
Domain 5. Exposure on	Metric 7	Experimental System/Test Media	Low	Test media preparation details were not provided other than DEHP was dissolved in					
		Preparation	2011	dimethyl sulfoxide. Concentrations were not measured.					
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.					
		Administration	8						
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.					
		Concentration							
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration (6 months) was appropriate to examine mechanistic effects.					
	Metric 11:	Number of Exposure Groups/	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1					
		Spacing of Exposure Levels		mg/L of DEHP, 0.5 mg/L of DEHP.					
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were near the solubility limit for DEHP, but an appropriate sol- vent was used. The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected be- tween the solvent and test substance).					
Domain 4: Tast Organia	m								
Domain 4: Test Organisi	Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the exposure was as follows: "Embryos were collected from the abdomens of healthy females.					
				and the larvae hatched within one week were used for the subsequent exposure experi- ment."					
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.					
		Conti	nued on next pa	nge					

Diethylhexyl Phthalate

		continu	ued from previ	ous page				
Study Citation: Duration: Exposure Route, Madia Path:	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquatic Toxicology 146:115-126. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Tava Species Age	Vertebrate: F	Fish: Orvias melastiama: Adult						
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2519010							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Low	50 fish per replicate were used, and there were 3 replicates per treatment group.For VTG analyses 5 fish livers were pooled. Pooling replicates is not best practice because it makes it impossible to capture variation across individual replicate animals.For RNA 3 replicates were used, which is a very small sample size for gene expression work. For E2 and T concentrations, 5 individuals per sex were used.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Housing and environmental conditions were appropriate for the marine medaka. The fish were raised in artificial seawater under standard laboratory conditions of 28 ± 1 °C on a 14:10 light/dark photoperiod. The fish were fed with freshly hatched Artemia nauplii twice daily.				
	Metric 17:	Outcome Assessment Methodology	High	The endocrine endpoints examined (E2 and testosterone concentrations, expression of hypothalamic pituitary gonadal axis genes as well as VTG) were sensitive and appropri- ate.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment methods were reported and were the same across groups.				
Domain 6: Confounding	y / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Information that could be used to compare environmental conditions across groups (measured concentrations, monitoring data for pH, DO, etc.) was limited.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested differences occurred that were unrelated to exposure.				
Damain 7. Data Dreamt								
Domain /: Data Present	Metric 21.	ysis Statistical Methods	High	The authors used one-way ANOVAs and Tukay's post has tests				
	Metric 22.	Reporting of Data	High	Means and SEM for both treatments and the control were reported				
	Metric 22.	Explanation of Unexpected Outcomes	High	The authors do not describe any unexpected outcomes				
Additional Comments:	The study ex evaluation for genes as wel	amines the effects of 6 months of exposure t form is relevant to the mechanistic effects ex l as VTG). The study does not verify exposure	o DEHP, and ev amined (E2 an re concentration	valuates a number of apical and mechanistic endpoints in the marine medaka. This d testosterone concentrations, expression of hypothalamic pituitary gonadal axis is.				

Study Citation: Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive									
	dysfunction	ysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquatic Toxicology 146:115-126.							
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: > 21	days						
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., chemica	al of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:									
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias melastigma; Adult							
Health Outcome:	Reproductiv	e/Teratogenic							
Chemical:	Di-ethylhex	yl phthalate (DEHP)							
HERO ID:	2519010								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.					
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the sub- stance identity was verified.					
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.					
Domain 2: Test Design	Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water					
	Metric 5:	Negative Control Response	High	The control responses were adequate					
	Metric 5:	Pandomized Allocation	Low	Passarahara did not stata if the fich ware allocated at random					
	Metric 0.	Kandoinized Anocation	LOW	Researchers did not state if the fish were anocated at random.					
Domain 3: Exposure Ch	aracterization								
Domain of Enposate of	Metric 7:	Experimental System/Test Media	Low	Only limited details were given regarding experimental system and test media prepa-					
		Preparation		ration methods. DEHP was dissolved in dimethyl sulfoxide. Concentrations were not measured.					
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.					
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.					
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate to examine reproductive effects (6 months).					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1 mg/L of DEHP, 0.5 mg/L of DEHP.					
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations are near the solubility limit for DEHP, but an appropriate solvent was used. The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected between the solvent and test substance).					
Domain 4: Test Organis	m		T						
	Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the ex- posure was as follows: "Embryos were collected from the abdomens of healthy females, and the larvae hatched within one week were used for the subsequent exposure experi- ment."					
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.					
		Conti	nued on next pa	age					

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (Oryzias melastigma). Aquatic Toxicology 146:115-126. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media. Path: Taxa, Species, Age: Vertebrate; Fish; Oryzias melastigma; Adult **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 2519010 Domain Metric Rating Comments Number of Organisms and Metric 15: Medium 50 fish per replicate were used, and there were 3 replicates per treatment group. To examine reproductive effects, five exposed males or females per replicate were paired Replicates per Group with five unexposed females or males. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High Housing and environmental conditions were appropriate for the marine medaka. The fish were raised in artificial seawater under standard laboratory conditions of 28 \pm 1 °C on a 14:10 light/dark photoperiod. The fish were fed with freshly hatched Artemia nauplii twice daily. Metric 17: Outcome Assessment Methodology High The reproductive endpoints examined (time to start spawning, eggs/female/day, fertilization success (%), sex ratio (female: male); Table 2) were sensitive and appropriate. Metric 18: Consistency of Outcome High Outcome assessment methods were reported and were the same across groups. Assessment Domain 6: Confounding / Variable Control Low Metric 19: Confounding Variables in Test Information that could be used to compare environmental conditions across groups

Additional Comments:	The study examines the effects of 6 months of exposure to DEHP and evaluates a number of apical and mechanistic endpoints in the marine medaka. This
	evaluation form is relevant to the reproductive endpoints (Time of start spawning, eggs/female/day, fertilization success (%), Sex ratio (female: male)). The
	study does not verify exposure concentrations.

Medium

High

High

High

trol were reported.

(measured concentrations, monitoring data for pH, DO, etc.) was limited.

The authors used one-way ANOVAs and Tukey's post hoc tests.

The authors do not describe any unexpected outcomes.

No information suggested differences occurred that were unrelated to exposure.

Means and SEM of reproductive parameters (Table 2) for both treatments and the con-

Overall Quality Determination

Domain 7: Data Presentation and Analysis

Metric 20:

Metric 21:

Metric 22:

Metric 23:

Design and Procedures

Statistical Methods

Reporting of Data

Outcomes Unrelated to Exposure

Explanation of Unexpected Outcomes

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>sheepshead minnow (Cyprinodon variegatus)</i> ; Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 1316224					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The test material was identified, and the CASRN was given.		
	Metric 2:	Test Substance Source	High	The source was listed as EG&G Bionomics Aquatic Toxicology Laboratory in Ware- ham, Massachusetts. No other information about the source was given.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A negative control was used.		
	Metric 5:	Negative Control Response	High	No mortality was reported in the controls.		
	Metric 6:	Randomized Allocation	Medium	Test organisms were impartially distributed to each chamber (pdf pg 136).		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail and accounted for the properties of the test material. For all low-solubility phthalates, an enhanced mixing procedure was used, while for the butyl benzyl phthalate exposure, microbial degradation was accounted for with a cleaning pro- cedure that was implemented daily. The authors reported significant degradation of the test material throughout the test, but quantified the degradation throughout the test and reported the endpoint in terms of mean-measured concentration, so this does not have an impact on the results.		
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	High	Concentrations were measured using GS-MS to account for poor water solubility.		
	Metric 10:	Exposure Duration and Frequency	High	A 96 hour exposure was appropriate for an acute test.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Test concentration spacing was limited, as this was designed as a limit test up to the solubility limit of the chemical.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit, which were reported in Appendix A (pdf pg 164).		
Domain 4: Test Organis	m					
C C	Metric 13:	Test Organism Characteristics	High	Specimens were either cultured at the Laboratory orpurchased commercially. All fish were tested as juveniles, <10weeks old.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	A 96 hour acclimation period was reported.		
Continued on next page						

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Diethylhexyl Phthalate

HERO ID: 1316224 Table: 1 of 1

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>sheepshead minnow (Cyprinodon variegatus</i>); Juvenile Mortality Di-ethylhexyl phthalate (DEHP) 1316224					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Only two replicates of 10 fish were used in each treatment.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and biomass loading was appropriate.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes(s) of interest (mortality).		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The study reported minor differences among the study groups with respect to environ- mental conditions or other non-treatment-related factors, but these are unlikely to have a substantial impact on results. Authors reported that dissolved oxygen fell below guide- line recommended levels, and control mortality was high for one phthalate (not this phthalate). As no mortalities were observed in any test concentrations for this chemical, this was not determined to affect this test.		
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure (e.g., infec- tion) were reported for each study group, and there were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	No mortality was reported, so no statistical analysis was needed.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported quantitatively.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily ex- plained. Low DO did not have an affect on the outcome, and high mortality in controls was not reported for this chemical.		
Additional Comments:	The study re 1C, butyl ber	port for the Sheepshead minnow test begir nzyl phthalate was referred to as 1D, DIDP	ns on pg 124 was referred	of the PDF. DEHP is referred to as phthalate 1H, dibutyl phthalate was referred to as 1 to as 1L, and DINP was referred to as 1J.		

Overall Quality Determination High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Liu, Y., Guan, Y., Yang, Z., Cai, Z., Mizuno, T., Tsuno, H., Zhu, W., Zhang, X. (2009). Toxicity of seven phthalate esters to embryonic development of the abalone Haliotis diversicolor supertexta. Ecotoxicology 18(3):293-303. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 607762					
Domain	071102	Metric	Rating	Comments		
Domain 1: Test Substan	ce	Methe	Runng	Comments		
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	Low Low High	The chemical was identified only by name. No other information was provided. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as at least 99%.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4: Metric 5:	Negative Controls Negative Control Response	High High	Study authors reported using appropriate concurrent negative and solvent control groups. The biological responses (percentage of cleavage, normal blastula, larval settlement and matemorphosic) of the percentage control groups were adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization Metric 7:	Experimental System/Test Media	Medium	Methods for preparation of the test media were described in adequate detail: however.		
		Preparation		steps taken to minimize loss of test substance were not reported.		
	Metric 8:	Consistency of Exposure Administration	High	The exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at the end of the experiment, but it was not clear whether new test solutions were used for the incubation from the swimming stage to the metamorphosis stage. Measured concentrations were similar to nominal concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	The total duration of the exposure was 96 hours. The experiment was done in 2 phases covering developmental stages from fertilization to early veliger stage (12 hours) and from veliger to metamorphosis stage (84 hours).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and the spacing of exposure levels. 96 hr EC-50 values could not be established using the concentrations tested.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration (3.75 %v/v) slightly exceeded an appropriate concentration, but the biological response of the solvent control was acceptable.		
Domain 4. Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the choice of the test species source. Test organisms were collected from the field, and prior exposure to phthalates may have occurred.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.		
Continued on next page						

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Diethylhexyl Phthalate

continued from previous page								
Study Citation: Duration:	Liu, Y., Guar abalone Halio Overall Dura	Liu, Y., Guan, Y., Yang, Z., Cai, Z., Mizuno, T., Tsuno, H., Zhu, W., Zhang, X. (2009). Toxicity of seven phthalate esters to embryonic development of the abalone Haliotis diversicolor supertexta. Ecotoxicology 18(3):293-303. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Media, Path:	Aquatic (mar	rine); Water; Not determined by study autho	rs (i.e., chemical	l of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome:	Invertebrate; Development	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo Development/Growth						
Chemical: HERO ID:	Di-ethylhexyl phthalate (DEHP) 697762							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and the number of replicates were reported. The fertilized egg density used for each treatment group and controls were not verified after adding 10 ml of fertilization medium to each test vessel.				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to the maintenance of organism health. DEHP was found in the dilution medium $(1,119 \pm 20 \text{ ng/l}$; Table 2).				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes. The percentage of embryos undergoing cleavage and the percentage of settled larvae were not sensitive endpoints. However, the percentage of normal blastula was a sensitive end point, and 9hr EC 50 values were calculated. 96 hr -EC 50 values based percentage of larvae that underwent metamorphosis could not be calculated, instead 96- hr NOEC values were reported.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	/ Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.				
Domain 7: Data Presenta	ation and Analy	vsis						
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. ANOVA was used to test for differences among treatments, and probit analysis was used to calculate 9-hr EC 50 values.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unex- pected outcomes. There was large within study variability for the settlement end point as both normal and abnormal larvae settled.				

Additional Comments: 9hr EC 50 values were reported based on the percentage of normal blastula, and 96-hr NOEC values were reported based on the percentage of larvae that underwent metamorphosis. For DEHP, 96-hr NOEC values were adjusted for background concentration of DBP in the dilution medium.

Overall Quality Determination

Study Citation:	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta.					
Duration: Exposure Route, Media, Path:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Mollusks; Haliotis diversicolor supertexta	; Larvae			
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1322103					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce	—				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich China.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as >99%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.		
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were reported for settlement rate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail, but uncertainty over duration and concern for chemical loss caused downgrading.		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was to the trochophore stage, which was somewhat arbitrary.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. A solvent aided in dissolution.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.		
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.		
		Conti	inued on next pa	ge		
			Dage 502 of 050	2		

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1322103 Table: 1 of 1

. H., Zhang, X. J., Cai, Z. H. (2009). Toxic eff Journal of Oceanology and Limnology 27(2): Duration: 0 - 4 days (0-96h); Exposure Durati (marine); Water; Not determined by study autorate; Mollusks; <i>Haliotis diversicolor supertex</i> ment/Growth (hexyl phthalate (DEHP) 3 <u>Metric</u> 17: Outcome Assessment Methodology 18: Consistency of Outcome Assessment	ects of several pht 395-399. on: 0 - 4 days (0-9 chors (i.e., chemica <i>ta</i> ; Larvae Rating High High	halate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta. 96h) al of interest in exposure water, but unable to determine exact uptake route) <u>Comments</u> The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
Journal of Oceanology and Limnology 27(2): Duration: 0 - 4 days (0-96h); Exposure Durati (marine); Water; Not determined by study autorate; Mollusks; <i>Haliotis diversicolor supertex</i> ment/Growth Ihexyl phthalate (DEHP) 3 <u>Metric</u> 7: Outcome Assessment Methodology [8: Consistency of Outcome Assessment	395-399. on: 0 - 4 days (0-9 hors (i.e., chemica ta; Larvae Rating High High	96h) al of interest in exposure water, but unable to determine exact uptake route) Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
Duration: 0 - 4 days (0-96h); Exposure Durati (marine); Water; Not determined by study au rate; Mollusks; <i>Haliotis diversicolor supertex</i> oment/Growth lhexyl phthalate (DEHP) 3 <u>Metric</u> 7: Outcome Assessment Methodology 8: Consistency of Outcome Assessment	on: 0 - 4 days (0-9 hors (i.e., chemica ta; Larvae Rating High High	26h) al of interest in exposure water, but unable to determine exact uptake route) Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
(marine); Water; Not determined by study autorate; Mollusks; <i>Haliotis diversicolor supertex</i> oment/Growth hexyl phthalate (DEHP) 3 <u>Metric</u> 17: Outcome Assessment Methodology 18: Consistency of Outcome Assessment	hors (i.e., chemica ta; Larvae Rating High High	Comments Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
rate; Mollusks; <i>Haliotis diversicolor supertex</i> oment/Growth lhexyl phthalate (DEHP) 3 <u>Metric</u> 17: Outcome Assessment Methodology 18: Consistency of Outcome Assessment	ta; Larvae Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
rate; Mollusks; <i>Haliotis diversicolor supertex</i> oment/Growth lhexyl phthalate (DEHP) 3 <u>Metric</u> 7: Outcome Assessment Methodology 8: Consistency of Outcome Assessment	ta; Larvae Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
ment/Growth hexyl phthalate (DEHP) 3 Metric 17: Outcome Assessment Methodology 8: Consistency of Outcome Assessment	Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
Ihexyl phthalate (DEHP) 3 Metric 17: Outcome Assessment Methodology 8: Consistency of Outcome Assessment	Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
3 Metric 17: Outcome Assessment Methodology 18: Consistency of Outcome Assessment	Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
Metric 7: Outcome Assessment Methodology 8: Consistency of Outcome Assessment	Rating High High	Comments The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
 7: Outcome Assessment Methodology 8: Consistency of Outcome Assessment 	High High	The outcome assessment methodology addressed the intended outcome of interest. The outcome assessment protocol was reported and consistent across study groups.
8: Consistency of Outcome Assessment	High	The outcome assessment protocol was reported and consistent across study groups.
Assessment		
e Control		
9. Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
Design and Procedures	mgn	There were no reported anterences among the study groups in environmental conditions
20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes
		unrelated to exposures.
Analysis		
Anarysis 21. Statistical Methods	Low	Statistical analysis was performed but not described adequately
22: Reporting of Data	High	Data for exposure related findings were presented for each treatment and control group
22. Explanation of Unavpacted Outcomes	High	There were no unexposed outcomes
25. Explanation of Unexpected Outcomes	nigii	There were no unexpected outcomes.
	20: Outcomes Unrelated to Exposure Analysis 21: Statistical Methods 22: Reporting of Data 23: Explanation of Unexpected Outcomes	20: Outcomes Unrelated to Exposure Medium I Analysis 21: Statistical Methods Low 22: Reporting of Data High 23: Explanation of Unexpected Outcomes High

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Study Citation:	Zhou, J., Ca	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ-					
Duration:	mental Pollu Overall Dura	tion 159(5):1114-1122. ation: 4 - 10 days: Exposure Duration: 4 -	10 days				
Exposure Route,	Aquatic (ma	rine): Water: Not determined by study aut	nors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:			(,				
Taxa, Species, Age:	Invertebrate;	Mollusks; Haliotis diversicolor supertext	a; Embryo				
Health Outcome:	Mechanistic	-Cell signaling/function	-				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1249532						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The purity was reported as $\geq 98\%$.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.			
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Demain 2. Europeuro Champatorization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de			
	Wieute 7.	Preparation	mgn	scribed in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered			
		Administration	0	consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
		Concentration					
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit, but they were aided by a solvent.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
		Conditions	C				
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and the number of replicates were reported and sufficient			
		Replicates per Group		to characterize toxicological effects.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conductive to the maintenance of organism health.			
		Cont	inued on nev	t nage			
Continueu on next page							

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Environmental Hazard Evaluation

HERO ID: 1249532 Table: 1 of 3

		contir	nued from p	previous page	
Study Citation:	Zhou, J., Ca	i, Z. H., Xing, K. Z. (2011). Potential mech	nanisms of p	hthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ-	
	mental Pollu	tion 159(5):1114-1122.			
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days		
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study authors	ors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Invertebrate; Mollusks; Haliotis diversicolor supertexta; Embryo				
Health Outcome:	Mechanistic-Cell signaling/function				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1249532				
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.	
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed	
		Assessment		consistently across study groups.	
Domain 6: Confounding	v / Variable Co	atrol			
Domain 0. Comounding	Metric 10	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions	
	Methe 17.	Design and Procedures	mgn	There were no reported differences among the study groups in environmental conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
		<u> </u>	6	66.1	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This evaluati	ion is for gene expression.			
		6 1			
Overall Qualit	ty Detern	nination	High		

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HERO ID: 1249532 Table: 2 of 3

Study Citation:	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ-					
Duration: Exposure Route, Media, Path:	Mental Pollution 159(5):1114-1122. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	; Mollusks; Haliotis diversicolor supertext	a; Embryo			
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1249532					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT' 1			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	LOW	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	The purity was reported as >=98%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.		
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-		
		Preparation		scribed in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported, and exposures were administered		
	Matria O.	Administration	τ	consistently across study groups.		
	Metric 9:	Concentration	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit. They were aided by a solvent.		
Domain 4: Test Organis	m					
2 sinum 1. rest organis	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
		Conditions	0	,		
	Metric 15:	Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient		
		Replicates per Group		to characterize toxicological effects.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conductive to the maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.		
Continued on next page						

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Diethylhexyl Phthalate

		contir	nued from p	previous page			
Study Citation:	Zhou, J., Ca	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ-					
	mental Pollu	tion 159(5):1114-1122.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days				
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Mollusks; Haliotis diversicolor supertexta; Embryo						
Health Outcome:	Development/Growth						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1249532						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups.			
Domain 6. Confoundin	a / Variabla Ca	ates					
Domain 0. Comoundin	g / Vallable Col Matria 10:	Confounding Variables in Test	Uich	These wars no concreted differences among the study around in any incompartal conditions			
	Metric 19:	Confounding variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Matria 20.	Design and Procedures Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment			
	Metric 20.	Outcomes Onielated to Exposure	Tiigii	There were no unreferces among groups that could influence the outcome assessment.			
Domain 7: Data Presen	tation and Anal	ysis					
	Matria 21.	Statistical Methods	High	Statistical methods were clearly described.			
	Metric 21:	branbrieta nietaloto	Ų	•			
	Metric 21: Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1249532 Table: 3 of 3

ental Polluti erall Durat uatic (mari vertebrate; l echanistic-C -ethylhexyl 49532	ion 159(5):1114-1122. ion: 4 - 10 days; Exposure Duration: 4 - ine); Water; Not determined by study auth Mollusks; <i>Haliotis diversicolor supertexta</i> Oxidative stress (including redox biology) phthalate (DEHP)	10 days hors (i.e., che a; Embryo)	mical of interest in exposure water, but unable to determine exact uptake route)					
vertebrate; 1 echanistic-C -ethylhexyl 49532	Mollusks; <i>Haliotis diversicolor supertexta</i> Oxidative stress (including redox biology) phthalate (DEHP)	a; Embryo)						
echanistic-C -ethylhexyl 49532	Oxidative stress (including redox biology) phthalate (DEHP))						
-ethylhexyl 49532	phthalate (DEHP)		Di-ethylhexyl phthalate (DEHP)					
49532	Mataia	1249532						
	Natria Dating Comments							
	метс	Rating	Comments					
. • 1		TT: 1						
etric 1:	Test Substance Identity	High	The chemical was identified by name.					
etric 2:	Test Substance Source	LOW	The test substance identity was not analytically verified by the performing laboratory.					
erric 5:	Test Substance Purity	High	The purity was reported as >=98%.					
etric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.					
etric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.					
etric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.					
erization								
etric 7:	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de-					
	Preparation	TT: -1-	scribed in adequate detail.					
etric 8:	A dministration	High	Details of exposure administration were reported, and exposures were administered					
etric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured					
	Concentration	Low	Exposure concentrations were not inclustred.					
etric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.					
etric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate.					
etric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit, but they were aided by a solvent.					
etric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.					
etric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.					
etric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.					
	Repleates per Group							
nent								
etric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conductive to the maintenance of organism health.					
etric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.					
	tric 2: tric 3: tric 4: tric 5: tric 6: erization tric 7: tric 8: tric 9: tric 10: tric 11: tric 12: tric 13: tric 14: tric 15: ent tric 16: tric 17: tric 17:	tric 2: Test Substance Source tric 3: Test Substance Purity tric 4: Negative Controls tric 5: Negative Control Response tric 6: Randomized Allocation erization tric 7: Experimental System/Test Media Preparation tric 8: Consistency of Exposure Administration tric 9: Measurement of Test Substance Concentration tric 10: Exposure Duration and Frequency tric 11: Number of Exposure Groups/ Spacing of Exposure Levels tric 12: Testing at or Below Solubility Limit tric 13: Test Organism Characteristics tric 14: Acclimatization and Pretreatment Conditions tric 15: Number of Organisms and Replicates per Group tent tric 17: Outcome Assessment Methodology	tric 2: Test Substance Source Low tric 3: Test Substance Purity High tric 4: Negative Controls High tric 5: Negative Control Response High tric 6: Randomized Allocation Low erization tric 7: Experimental System/Test Media High Preparation tric 8: Consistency of Exposure High Administration tric 9: Measurement of Test Substance Low Concentration tric 10: Exposure Duration and Frequency High tric 11: Number of Exposure Groups/ High Spacing of Exposure Levels tric 12: Testing at or Below Solubility Limit Low tric 13: Test Organism Characteristics Low tric 14: Acclimatization and Pretreatment High Conditions tric 15: Number of Organisms and Medium Replicates per Group tent tric 17: Outcome Assessment Methodology High					

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Diethylhexyl Phthalate

HERO ID: 1249532 Table: 3 of 3

	continued from previous page						
Study Citation:	Zhou, J., Cai mental Pollu	hou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ- nental Pollution 159(5):1114-1122.					
Duration:	Overall Dura	tion: 4 - 10 days; Exposure Duration: 4 - 10	0 days				
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Mollusks; Haliotis diversicolor supertexta; Embryo					
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1249532						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups.			
Domain 6: Confounding / Variable Control							
-	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	-				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Prasant	ation and Anal	Nois					
Domain 7. Data Mesent	Metric 21.	Statistical Methods	High	Statistical methods were clearly described			
	Metric 21.	Reporting of Data	High	Data for exposure related findings were presented for each treatment and control group			
	Metric 22.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
	Meule 25.	Explanation of Onexpected Outcomes	mgn	There were no unexpected outcomes.			
Additional Comments:	This evaluati	on is for MDA and POD changes in the org	anisms.				
Overall Quality Determination High							

continued from previous page

Study Citation:	Yang, Z. H.,	Zhang, X. J., Cai, Z. H. (2009). Toxic effect	cts of several phth	alate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta.
Duration: Exposure Route, Media, Path:	Chinese Jou Overall Dura Aquatic (ma	rnal of Oceanology and Limnology 27(2):3 ation: Not-reported; Exposure Duration: No rine); Water; Not determined by study auth	95-399. ot-reported ors (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate	; Mollusks; Haliotis diversicolor supertexta	; Embryo	
Health Outcome:	Developmen	nt/Growth		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	1322103			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	High	The test substance source was Sigma-Aldrich China.
	Metric 3:	Test Substance Purity	High	The test substance purity was reported as >99%.
Domain 2: Test Design				
e	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were reported for abnormal development.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail, but uncertainty over duration and concern for chemical loss caused downgrading.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was to the trochophore stage, which was somewhat arbitrary.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. A solvent aided in the dissolution of the test substance.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.
		Conti	inued on next pa	nge
			Page 601 of 95	8

Diethylhexyl Phthalate

		conti	nued from previ	ous page			
Study Citation:	Yang, Z. H., Chinese Jour	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta. Chinese Journal of Oceanology and Limnology 27(2):395-399.					
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: No	ot-reported				
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Mollusks; Haliotis diversicolor supertexta	; Embryo				
Health Outcome:	Developmen	t/Growth	-				
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	(D: 1322103						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes of interest; not all organisms were examined.			
	Metric 18:	Consistency of Outcome	Low	Somewhat subjective assessments were made. The term "abnormal" was not well de-			
		Assessment		fined.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups from out- comes unrelated to exposures.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Reporting of Data	Low	The term "abnormal" was not well defined. It was unclear which abnormalities were			
				assessed.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						

Overall Quality Determination

aponicus. Fish and Shellfish Immunol 4 - 10 days; Exposure Duration: 4 - 1 Water; Not determined by study author usks; <i>Macrophthalmus japonicus</i> ; Not halate (DEHP) Metric t Substance Identity t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response hdomized Allocation	logy 87:322-332 0 days ors (i.e., chemica t Applicable (e.g Rating Low Low High High High High Low	Al of interest in exposure water, but unable to determine exact uptake route) at, fungi or algae studies) or Not Reported Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Water; Not determined by study authors usks; Macrophthalmus japonicus; Not nalate (DEHP) Metric t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Rating Low Low High High High Low	al of interest in exposure water, but unable to determine exact uptake route) g., fungi or algae studies) or Not Reported Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Mater, For determined by study data usks; <i>Macrophthalmus japonicus</i> ; Not malate (DEHP) <u>Metric</u> t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response adomized Allocation	Rating Low Low High High High Low	Comments Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
usks; <i>Macrophthalmus japonicus</i> ; Not halate (DEHP) <u>Metric</u> It Substance Identity It Substance Source It Substance Purity gative Controls gative Control Response hdomized Allocation	Applicable (e.g Rating Low Low High High High Low	Comments Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Metric Metric t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Rating Low Low High High High Low	Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Metric Metric t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Rating Low Low High High High Low	Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Metric t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Rating Low Low High High High Low	Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
Metric t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Rating Low Low High High High Low	Comments The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Low Low High High High Low	The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
t Substance Identity t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Low Low High High High Low	The chemical was identified by name only. The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
t Substance Source t Substance Purity gative Controls gative Control Response ndomized Allocation	Low High High High Low	The test substance identity was not analytically verified by the performing laboratory. The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
t Substance Purity gative Controls gative Control Response ndomized Allocation	High High High Low	The chemical purity was reported as 99%. Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
gative Controls gative Control Response ndomized Allocation	High High Low	Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
gative Controls gative Control Response ndomized Allocation	High High Low	Study authors reported using an appropriate concurrent negative control group. The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
gative Control Response ndomized Allocation	High Low	The biological response of the negative control group was suitable. Researchers did not report how organisms were allocated to study groups.
ndomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
perimental System/Test Media	Low	The experimental system was not described well. The study provided only limited de-
paration	Medium	tails on the measures taken to appropriately prepare the test concentrations.
ministration	Medium	rew details of the exposure administration were reported. The solvent concentration used was not reported
asurement of Test Substance	Low	Exposure concentrations were not measured
ncentration	Low	Exposure concentrations were not inclusived.
posure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
mber of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were suitable for a
acing of Exposure Levels	-	dose response.
ting at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the biological response of the solvent control was reported and adequate.
t Organism Characteristics	Low	There were concerns regarding the source of the test organisms. Crabs were collected
	20	from fish markets, and prior exposure to DEHP may have occurred.
climatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
nditions	Madimu	
mber of Organisms and	Medium	I ne number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects
	nber of Exposure Groups/ cing of Exposure Levels ting at or Below Solubility Limit t Organism Characteristics climatization and Pretreatment aditions nber of Organisms and blicates per Group	mber of Exposure Groups/ High cing of Exposure Levels Medium ting at or Below Solubility Limit Medium t Organism Characteristics Low elimatization and Pretreatment High nditions Medium mber of Organisms and Medium

Continued on next page ...

Diethylhexyl Phthalate

Study Citation:							
Study Citation.	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, Macrophthalmus japonicus. Fish and Shellfish Immunology 87:322-332.						
Duration:	Overall Dura	tion: 4 - 10 days; Exposure Duration: 4 - 10) days				
Exposure Route,	Aquatic (mai	rine); Water; Not determined by study autho	rs (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Mollusks; Macrophthalmus japonicus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5567571						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were ade- quate and whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly described.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	/ Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	One-way analysis of variance (ANOVA) was conducted combined with Tukey's multiple range test.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Environment markets and	al conditions and exposure concentrations v cultured in natural seawater. Background co	were not measure ncentration of D	ed/reported during the 7 day experiment. Test organism were collected from fish EHP in dilution medium was not reported.			

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 5567571 Table: 2 of 2

Study Citation:	Park, K., Ki Macrophthal	m, W. S., Kwak, I. S. (2019). Endocrine-dia mus japonicus. Fish and Shellfish Immuno	srupting chemica	Is impair the innate immune prophenoloxidase system in the intertidal mud crab,			
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (ma	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic Di-ethylhexy 5567571	Mollusks; <i>Macrophthalmus japonicus</i> ; Not Biomarkers (exposure and effect) I phthalate (DEHP)	t Applicable (e.g	., fungi or algae studies) or Not Reported			
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		Ŧ				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	LOW	The chamical purity was not analytically verified by the performing laboratory.			
	Metric 5:	Test Substance Purity	nigii	The chemical purity was reported as 99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system was not described well. The study provided only limited de- tails on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Medium	Few details of the exposure administration were reported. The solvent concentration used was not reported.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the biological response of the solvent control was reported and adequate.			
Domain 4. Test Organis	m						
Zomani i. 10st Organis.	Metric 13:	Test Organism Characteristics	Low	There were concerns regarding the source of the test organisms. Crabs were collected from fish markets, and prior exposure to DEHP may have occurred.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were ade- quate and whether differences occurred between control and exposed populations.			
		Conti	nued on next pa	ge			

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 5567571 Table: 2 of 2

		contin	ued from previ	ous page			
Study Citation: Duration: Exposure Route, Madia Bathy	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, Macrophthalmus japonicus. Fish and Shellfish Immunology 87:322-332. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Tava Species Age	Invertebrate: Mollusks: Macrophthalmus japonicus: Not Applicable (e.g. fungi or algae studies) or Not Reported						
Health Outcome	Mechanistic-Riomarkers (exposure and effect)						
Chemical:	Di-ethylhexy	Di-ethylbexyl phthalate (DEHP)					
HERO ID:	5567571						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. Methods for phenoloxidase activity and proPO-related gene expression were given in detail.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. One-way analysis of variance (ANOVA) was con- ducted combined with Tukey's multiple range test.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Phenoloxida (LGBP), pro creas of inter	se activity, mRNA transcript, and activity lev PO, phenoloxidase (PO), peroxinectin (PE), rtidal mud crabs.	vels of six imm serine protease	ine-related genes, including lipopolysaccharide and β -1,3-glucan-binding protein inhibitor (Serpin), and trypsin (Tryp), were assessed in gill and in the hepatopan-			

Study Citation:	Adams, W. J	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574				
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (ma	ition: 0 - 4 days (0-96h); Exposure Duration in the study authorized by study authoriz	bor: $0 - 4$ days	(0-96h) mical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate;	Arthropods; Mysidopsis bahia; Not Appli	icable (e.g., fi	ingi or algae studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		_			
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.		
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.		
	Metric 3:	Test Substance Purity	High	There was at least 95% purity.		
Domain 2: Test Design						
C C	Metric 4:	Negative Controls	High	A negative control was reported.		
	Metric 5:	Negative Control Response	High	The control response was acceptable.		
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures taken to prevent volatilization were not reported.		
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.		
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of the exposure were appropriate for the test.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.		
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.		
Domain 4: Test Organis	m					
in rest organis	Metric 13:	Test Organism Characteristics	Low	The source was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation period for the test was reported.		
		Conditions	U			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.		
Domain 5: Outcome As	sessment					

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		conti	nued from p	revious page			
Study Citation:	Adams, W. J	., Biddinger, G. R., Robillard, K. A., Gors	uch, J. W. (1	995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic			
	organisms. E	Environmental Toxicology and Chemistry 1	4(9):1569-13	574.			
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)			
Exposure Route,	Aquatic (ma	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Mysidopsis bahia; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.			
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent across groups.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.			
		Design and Procedures	e				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.			
Domain 7: Data Present	ation and Anal	vsis					
2 chiuni / Putu i loson	Metric 21.	Statistical Methods	High	Statistical methods were performed and described			
	Metric 22	Reporting of Data	Medium	Only treatment endpoints were reported			
	Metric 22:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported			
	metric 23.	Explanation of Onexpected Outcomes	mgn				
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Bionomics,,	EG&G (1984). Acute toxicity of twelve pl	hthalate ester	s to mysid shrimp (Mysidopsis bahia).		
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	(0-96h)		
Exposure Route,	Aquatic (ma	rine), Aquatic (brackish); Water; Not dete	rmined by stu	dy authors (i.e., chemical of interest in exposure water, but unable to determine exact		
Media. Path:	uptake route)	2			
Taxa, Species, Age:	Invertebrate:	Arthropods: <i>Mysidopsis bahia</i> : Juvenile				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1316220	- p				
Domain	1310220	Matric	Dating	Comments		
Domain 1: Tast Substan	22	Wethe	Kating	Comments		
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No CASRN or structure were		
	Metric 2:	Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA)		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the substance were not included in the study.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Authors reported using negative controls.		
	Metric 5:	Negative Control Response	High	The response of the negative controls was adequate.		
	Metric 6:	Randomized Allocation	Medium	Mysid shrimp were maintained 1-3 days before they were distributed into test vessels.		
				J		
Domain 3: Exposure Ch	aracterization					
ľ	Metric 7:	Experimental System/Test Media Preparation	High	The experimental design followed protocol guidelines.		
	Metric 8:	Consistency of Exposure	High	Authors reported consistent administration.		
	Metric 9:	Administration Measurement of Test Substance	High	Phthalates were analytically verified and measured.		
	Metric 10:	Exposure Duration and Frequency	High	The test duration followed protocol		
	Metric 11:	Number of Exposure Groups/	Low	This study only performed a single exposure (0.44 mg/L) as a range finding test re-		
		Spacing of Exposure Levels	2011	ported no effect at levels below the water solubility limit.		
	Metric 12:	Testing at or Below Solubility Limit	Low	The single concentration used for DEHP was 0.44 mg/L and is over the solubility listed in the Final Scope (0.27 mg/L).		
Domain 4: Test Organis	m					
8	Metric 13:	Test Organism Characteristics	Medium	The source of the organisms was reported. Details beyond that were not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were housed for 1-3 days prior to treatment.		
		Conditions				
	Metric 15:	Number of Organisms and	Medium	Replicates followed protocol.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	High	Environmental conditions were adequate and described in detail		
	Metric 17	Outcome Assessment Methodology	High	Outcomes were reported and addressed		
		~		· · · · · · · · · · · · · · · · · · ·		
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		conti	nued from p	revious page			
Study Citation:	Bionomics,,	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (ma	Aquatic (marine), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Media, Path:	uptake route	uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Mysidopsis bahia; Juvenile					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1316220						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed and reported.			
		Assessment					
Domain 6: Confounding	, / Variable Cou	ntrol					
Domain of Comountaing	Metric 19	Confounding Variables in Test	High	No differences were reported			
	metile 19.	Design and Procedures	mgn	no americaes were reported.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.			
Domain /: Data Present	ation and Anal	ysis	Ŧ				
	Metric 21:	Statistical Methods	Low	No data analysis was presented as the LC50 was >0.44 mg/L.			
	Metric 22:	Reporting of Data	High	Data was reported adequately.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	Based on pre included in t	eliminary studies, concentrations of DEHP he final study.	below the so	blubility limit did not result in adverse outcomes; therefore, a single concentration was			
Overall Quali	ty Detern	nination	High				

Study Citation:	Brown, D., 7	Thompson, R. S. (1982). Phthalates and the	aquatic environm	nent: Part 2. The bioconcentration and depuration of di-2-ethylhexyl phthalate and
Duration:	diisodecyl pl Overall Dura	hthalate in mussels, (Mytilus edulis). Chemation: > 21 days; Exposure Duration: > 21	osphere 11(4):42 days	27-435.
Exposure Route, Modia, Bathy	Aquatic (ma	rine); Water; Not determined by study auth	ors (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate	Mollusks; Mytilus edulis; Adult		
Health Outcome:	ADME (biot	ransformation)		
Chemical:	Di-ethylhexy	vl phthalate (DEHP)		
HERO ID:	1334379			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce	Trad Calendary - Identity	Madian	
	Metric 1: Metric 2:	Test Substance Source	High	Correct nomenciature was given, and the specific form (radiolabeled) was reported. The source was reported. The radiolabled DEHP was synthesized (by Dr. D. Parker
	Metric 2.	Test Substance Source	mgn	Physics and Radioisotopes Services, ICI PIC,P 0 Box 2, Billingham, England) from phthalic anhydride, and the test substance identity was analytically verified by the per- forming laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as >97.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A concurrent negative solvent control was used.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
ľ	Metric 7:	Experimental System/Test Media Preparation	Medium	Experimental system and test media preparation details were adequately reported but did not completely account for physical-chemical properties. A flow-through system was used, and exposure concentrations were measured.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but methods were not described well.
	Metric 10:	Exposure Duration and Frequency	High	This was a 28 day test to determine BCF.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose dependent effect but to determine BCF.
	Metric 12:	Testing at or Below Solubility Limit	Low	The solvent concentration (0.5 ml/l) slightly exceeded the typical recommended value (0.1 ml/l; OCSPP 850.173: Fish BCF guidelines), and the biological response of the solvent control was not reported.
Domain 4: Test Organis	m			
-	Metric 13:	Test Organism Characteristics	Low	Mussels were collected locally, and mean shell length and tissue weight of a subset of samples were reported. There was significant concern regarding the source of the mussels. Locally collected mussels could have been exposed to phthalates and a multitude of other stressors prior to the experiment.
		Conti	inued on next pa	

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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 1334379 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation: Duration: Exposure Route, Modia Path:	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 2. The bioconcentration and depuration of di-2-ethylhexyl phthalate and diisodecyl phthalate in mussels, (Mytilus edulis). Chemosphere 11(4):427-435. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Toyo Species Age:	Invertebrate	Molluske: Mytilus adulis: Adult					
Hoalth Outcome:	ADME (biot	monusks, <i>Mynnus eanns</i> , Adun					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	1334379						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	80 mussels were exposed to the 2 concentrations of DEHP. Replicates were not reported			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions (pH, dissolved oxygen, salinity, etc.) were not reported to evaluate if adequate and whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The methods for tissue concentration analysis were not given in detail. Lipid content in mussels was not determined. Since phthalates are hydrophobic, lipid fraction in mussels should have been determined before and at the end of the uptake experiment and depuration period. This is a serious omission and likely to have a substantial impact on results.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
Domain 6: Confounding	/ Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	Low	Data presentation was inadequate. The uptake and depuration rate constants with confi- dence limits were not reported. Methods for calculating BCF were not given, and BCF values were reported without measures of variability.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Mussels wer Uptake and o	e collected locally and were not acclimatized depuration rate constants were not reported.	1. Tissue concer BCF calculation	trations in controls were not reported. Lipid content in mussels was not analyzed.			
Study Citation:	RB, Laughli	n, J. R., Neff, J. M., Hrung, Y. C., Goodwin	, T. C., Giam, C	C. S. (1978). The effects of three phthalate esters on the larval development of the			
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Duration: Exposure Route, Media, Path:	grass shrimp Overall Dura Aquatic (ma	grass shrimp Palaemonetes pugio (Holthuis). Water, Air, and Soil Pollution 9(3):323-336. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	; Arthropods; PALAEMONETES PUGIO; La	irvae				
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1333217						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylhexyl phthalate (DEHP)].			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the solvent control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
r	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Differences from nominal values varied considerably.			
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via gas chromatography, but measured values were not similar to nominal concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of chemical were noticed in test chambers.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were col- lected from the wild, with minimal characteristic information given: "Gravid female Palaemonetes pugio were collected from salt marshes at the eastern end of Galveston Island, Texas. Separate collections were made between June and October, 1976, for test- ing each phthalate ester. Previous observations have established that the overall health and viability of adults do not vary significantly during this time of year (Tatem et al., 1976)."			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.			
		Contin	nued on next pa	ge			

Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	RB, Laughli	RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp Palaemonetes pugio (Holthuis). Water Air and Soil Pollution 9(3):323-336.					
Duration:	Overall Dura	Dverall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (ma	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	1						
Taxa, Species, Age:	Invertebrate:	Invertebrate: Arthropods: PALAEMONETES PUGIO: Larvae					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	1333217	1333217					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confoundin	a / Variable Co	ntrol					
Domain 0. Comounam	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Some variability was considered to be attributed to incomplete dissolution of the test chemical.			

Additional Comments: None

Overall Quality Determination

Study Citation:	RB, Laughli	RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the					
Duration: Exposure Route, Media. Path:	grass shrimp Overall Dura Aquatic (ma	Palaemonetes pugio (Holthuis). Water, Air, ttion: > 21 days; Exposure Duration: > 21 d rine); Water; Not determined by study author	and Soil Polluti lays rs (i.e., chemica	on 9(3):323-336. I of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Mortality Di-ethylhexy	nvertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae Aortality Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1333217			2			
Domain Domain 1: Test Substan	<u></u>	Metric	Rating	Comments			
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylbexyl phthalate (DEHP)]			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the solvent control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations. Measured values differed from nominal values considerably.			
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via gas chromatography, but measured values were not similar to nominal concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of the chemical were noticed in the test chambers.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were col- lected from the wild, with minimal characteristic information given: "Gravid female Palaemonetes pugio were collected from salt marshes at the eastern end of Galveston Island, Texas. Separate collections were made between June and October, 1976, for test- ing each phthalate ester. Previous observations have established that the overall health and viability of adults do not vary significantly during this time of year (Tatem et al., 1976)."			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).			
		Contin	ued on next pa	ge			

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp Palaemonetes pugio (Holthuis). Water, Air, and Soil Pollution 9(3):323-336. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Exposure Route, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; PALAEMONETES PUGIO; Larvae **Health Outcome:** Mortality Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 1333217 Domain Metric Rating Comments Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Environmental conditions were not sufficiently reported to evaluate if they were adequate. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology reported the intended outcome of interest. Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low The study did not provide enough information to allow a comparison of environmental **Design and Procedures** conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Statistical analysis was performed but not described adequately. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes Low The study did not report any measures of variability. Some variability was considered to be attributed to incomplete dissolution of the test chemical. Additional Comments: None

Overall Quality Determination

Study Citation:	RB, Laughlingrass shrimp	RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp Palaemonetes pugio (Holthuis). Water, Air, and Soil Pollution 9(3):323-336.				
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (mar	tion: > 21 days; Exposure Duration: > 21 d rine); Water; Not determined by study author	lays rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; ADME (biot Di-ethylhexy 1333217	nvertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae ADME (biotransformation) Di-ethylhexyl phthalate (DEHP) 333217				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylhexyl phthalate (DEHP)].		
	Metric 2: Metric 3:	Test Substance Purity	Low	The source was not reported. The purity and/or grade of the test substance were not reported		
	Metric 5.	Test Substance Funty	Low	The purity and/of grade of the test substance were not reported.		
Domain 2: Test Design						
C C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.		
	Metric 5:	Negative Control Response	High	The biological response of the solvent control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
	metric /.	Preparation	Low	test concentrations. Differences from nominal values varied considerably.		
	Metric 8:	Consistency of Exposure	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of the chemical were noticed in test the chambers.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were col- lected from the wild, with minimal characteristic information given:"Gravid female Palaemonetes pugio were collected from salt marshes at the eastern end of Galveston Island, Texas. Separate collections were made between June and October, 1976, for test- ing each phthalate ester. Previous observations have established that the overall health and viability of adults do not vary significantly during this time of year (Tatem et al., 1976)."		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).		
		Contin	ued on next pa			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page		
Study Citation:	RB, Laughli	RB, Laughlin, J. R., Neff, J. M., Hrung, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp Palaemonates purio (Holthuis). Water, Air, and Soil Pollution 9(3):323–336.				
Duration:	Overall Dura	Dverall Duration: > 21 days: Exposure Duration: > 21 days				
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study autho	rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	1		~ /			
Taxa, Species, Age:	Invertebrate;	Arthropods; PALAEMONETES PUGIO; La	rvae			
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	1333217	1333217				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were ade- quate.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
		Design and Procedures		conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. Some variability was considered to be attributed to incomplete dissolution of the test chemical.		
Additional Comments:	This form is	for the ADME outcome with DEHP.				

Overall Quality Determination

Study Citation:	Heindler, F. I	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate				
Duration: Exposure Route, Media. Path:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Parvocalanus crassirostris; L	arvae			
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	3859142					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce		_			
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.		
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.		
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. Chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is $>98\%$.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Controls for the acute mortality experiment were reported on page 3/8		
	Metric 5:	Negative Control Response	Medium	Control survival for nauplii was reported as 82.6% with an SE of 5.1%.		
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported for Nauplii.		
Domain 3: Exposure Cha	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The acute bioassay was described as a static-renewal test and was detailed for adults in section 2.2.1 (page 2/8).		
	Metric 8:	Consistency of Exposure	High	There were 6 treatment concentrations, and they appeared to be consistently applied.		
	Metric 9:	Measurement of Test Substance	Low	The study does not measure the compound and reports nominal treatment concentra- tions.		
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr exposure duration for these zooplankton is appropriate.		
	Metric 11:	Number of Exposure Groups/	High	The acute bioassay used 6 concentrations with a control.		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit. The highest concentra- tion used on the acute bioassay with nauplii was 1953 ng/L. The final Scope for DEHP lists the solubility for this compound as 2700 ug/L.		
Domain 4: Test Organisr	n					
rest organisi	Metric 13:	Test Organism Characteristics	High	The acute bioassays on nauplii were from in house cultures.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.		
	Metric 15:	Number of Organisms and	Medium	The authors reported 3 replicates per treatment concentration and 60 nauplii per repli-		
		Replicates per Group				

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

		contin	ued from previ	ous page			
Study Citation:	Heindler, F. I microparticle	M., Alajmi, F., Huerlimann, R., Zeng, C., Neves and Di(2-ethylhexyl)phthalate on the cala	wman, S. J., Van noid copepod, F	nvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.			
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Parvocalanus crassirostris; Larvae						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	3859142						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 48 hr bioassay.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology resulted in the formation of a dose-response curve presented in Figure 2.			
	Metric 18:	Consistency of Outcome Assessment	High	Assessment within this acute bioassay appeared to be consistent among treatment and control groups.			
Domain 6: Confounding	/ Variable Co	atrol					
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Low	Environmental conditions were not reported to indicate if they caused any differences			
	Wetter 19.	Design and Procedures	Low	Environmental conditions were not reported to indicate it they caused any differences.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to exposure influenced outcome assessment.			
Domain 7: Data Present:	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. The authors did not report how the dose-response curve was estimated with survival data. LC 50 concentrations were presented in section 3.1, and a "sigmoidal model" was presented in Figure 2 (page 5/8).			
	Metric 22:	Reporting of Data	Low	Raw data for exposure-related findings were not shown for each treatment and control, but adjusted survival data for treatment groups were given in Figure 2. Control survival were reported as a 48 hr mean of the replicates.			

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Heindler, F. I microparticle Overall Dura Aquatic (mar Invertebrate; Mortality Di-ethylhexy 3859142	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult Mortality Di-ethylhexyl phthalate (DEHP) 3859142					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		_				
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.			
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.			
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product was >98%.			
Domain 2 [.] Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Controls for the acute mortality experiment were reported on page 3/8. Solvent controls were used for the two highest concentrations of the compound.			
	Metric 5:	Negative Control Response	Low	Section 3.1 and supplemental notes (Table S.2) do not report control survival for the adult acute exposures.			
	Metric 6:	Randomized Allocation	Medium	Random allocation was reported.			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The acute bioassay was described as a static-renewal test and was detailed for adults in section 2.2.1 (page 2/8).			
	Metric 8:	Consistency of Exposure Administration	Low	Half of the 6 highest concentrations contained a 4% ethanol carrier due to solubility concerns. The lowest three treatment concentrations did not contain a solvent carrier. Authors reported using both solvent and non-solvent controls but did not report control survival.			
	Metric 9:	Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentra- tions.			
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr exposure duration for these zooplankton is appropriate.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The acute bioassay used 6 concentrations with a control and solvent controls. Authors reported in section 3.1 (page 4/8) that there were no significant differences between treatments and controls for the adult exposures. LC 50 values could not be established at the tested concentrations.			
	Metric 12:	Testing at or Below Solubility Limit	Low	The highest concentration (5120 ug/L) in the acute bioassay was above the solubility and the, second highest concentration (2560 ug/L) was near the limit. The solubility for this compound is 2700 ug/L. The solvent concentration used (4%) exceeded the recommended concentration of 1ml/l, and the percent survival in the control group was not reported.			

Domain 4: Test Organism

Diethylhexyl Phthalate

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Study Citation:	Heindler, F. I microparticl	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylbexyl)phthalate on the calanoid copened. Parvocalanus crassifications. Ecotoxicology and Environmental Safety 141:298-305					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route.	Aquatic (ma	rine): Water: Not determined by study autho	rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)			
Media. Path:	i iquitio (iiiu						
Taxa, Species, Age:	Invertebrate:	Invertebrate; Arthropods; Parvocalanus crassirostris; Adult					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3859142						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	High	The acute bioassays on adults were from in house cultures.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and	Medium	The authors report 5 replicates per treatment concentration and 10 randomly selected			
		Replicates per Group		remaies per repricate.			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 48 hr bioassay.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	The assessment within this acute bioassay appeared to be consistent among treatment			
		Assessment		and control groups.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to exposure influenced outcome assessment.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	The authors did not report how the dose-response curve was estimated with survival data.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text. Control data were not reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability in survival are reported in section 3.1 as standard error (SE).			

Overall Quality Determination

Study Citation: Duration: Exposure Route.	Heindler, F. microparticl Overall Dura Aquatic (ma	M., Alajmi, F., Huerlimann, R., Zeng, C., Ne es and Di(2-ethylhexyl)phthalate on the cala ation: > 21 days; Exposure Duration: 4 - 10 arine): Water: Not determined by study author	wman, S. J., Var moid copepod, l days ors (i.e., chemica	nvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.		
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Aquatic (marine); water; Not determined by study autors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult Mechanistic-Epigenetics-Genotox (including DNA repair) Di-ethylhexyl phthalate (DEHP) 3859142					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.		
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.		
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is $>98\%$.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The controls are present and detailed in section 2.5 and in Figure 5.		
	Metric 5:	Negative Control Response	High	Control gene expression after 24 days (6 days of chemical treatment and 18 days recovery) is presented in Figure 5.		
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
	Wette 7.	Preparation	Low	the study provided only infined details of the field substance were not measured during the study. For the 6 day exposure, the treatment solutions were not renewed; they were moved to new water without the chemical.		
	Metric 8:	Consistency of Exposure Administration	High	This study only used one treatment concentration (0.1 ng/L), which was 10% of the 48hr LC50 for nauplii.		
	Metric 9:	Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentra- tions.		
	Metric 10:	Exposure Duration and Frequency	High	The study was for 24 days to allow for the zooplankton to produce multiple generations. The chemical exposure was for 6 days.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One treatment concentration (0.1 ng/L) was chosen to represent toxicity to 10% of the 48hr LC50 for nauplii.		
	Metric 12:	Testing at or Below Solubility Limit	High	0.1 ng/L is under solubility limits. The Final Scope for DEHP lists the solubility for this compound as 2700 ug/L.		
Domain 4: Test Organis	m					
5	Metric 13:	Test Organism Characteristics	High	The gene expression experiment was conducted with inhouse copepod cultures.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.		
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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 3859142 Table: 1 of 3

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Study Citation:	Heindler, F. microparticl	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate nicroparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.					
Duration:	Overall Dur	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Aquatic (ma	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Parvocalanus crassirostris; Adult					
Health Outcome:	Mechanistic	Mechanistic-Epigenetics-Genotox (including DNA repair)					
Chemical:	Di-ethylhex	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3859142						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	5 replicates per treatment group and per control group were used.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 24 day study.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology assessed gene expression relative to two house- keeping genes.			
	Metric 18:	Consistency of Outcome Assessment	High	Assessment within this gene expression experiment appeared to be consistent among treatment and control groups.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	Environmental conditions were not reported to indicate if they caused any differences.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment.			
Domain 7: Data Presen	itation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Statistical analysis was performed and described in section 2.6. Authors used z tests on bayesian z-scores to test for significance.			
	Metric 22:	Reporting of Data	High	Section 3.4 and Figure 5 detail 6 day and 24 day gene expression for H3 and Hsp70. Data were represented as mean log2 (fold change) in expression (±SE; n=3) of Hsp70-like and H3 in treatments compared to the expression of the same genes in the control groups.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability in gene expression relative to the control population are reported in section 3.4 as standard error (SE).			
Additional Comments:	H3 gene exp detected foll	pression was downregulated after 6 days of owing DEHP exposure.	f DEHP exposur	e but not after 18 days of recovery. No changes in Hsp70-like expression were			

Overall Quality Determination

Study Citation:	Heindler, F.	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate					
	micropartic	nicroparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.					
Duration:	Overall Dur	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days				
Exposure Route,	Aquatic (ma	arine); Water; Not determined by study authors	ors (i.e., chemica	al of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Arthropods; Parvocalanus crassirostris; N	ot Applicable (e.	g., fungi or algae studies) or Not Reported			
Health Outcome:	Other (pleas	se specify below) (Population Size)					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	3859142						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.			
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.			
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is $>98\%$.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Controls are present and detailed in section 2.4 and in Figure 1.			
	Metric 5:	Negative Control Response	High	Control population size after 24 days is presented in Figure 4. All treatment groups are compared as their % relative to the control population size at the end of the experiment.			
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.			
Domain 3: Exposure Ch	naracterization		_				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the concentrations of the test substance were not measured during the study. For the 6 day exposure, the treatment solutions were not renewed.			
	Metric 8:	Consistency of Exposure Administration	High	This study spanned over multiple generations (24 days) and only used one treatment concentration (0.11 ng/L) , which was 10% of the 48hr LC50 for nauplii.			
	Metric 9:	Measurement of Test Substance	Low	The study does not measure the compound and reports nominal treatment concentra- tions.			
	Metric 10:	Exposure Duration and Frequency	High	The study was 24 days to allow for the zooplankton to produce multiple generations.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One treatment concentration was (0.11 ng/L) chosen to represent toxicity to 10% of the 48hr LC50 for nauplii.			
	Metric 12:	Testing at or Below Solubility Limit	High	0.11 ng/L is under the solubility limit. The Final Scope for DEHP lists the solubility for this compound as 2700 ug/L.			
Domain 4: Test Organis	m						
5	Metric 13:	Test Organism Characteristics	High	The multigeneration experiment was conducted with inhouse copepod cultures.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates per treatment and per control group were used. There were 36 adults and 68 nauplii per replicate.			
Domain 5: Outcome As	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates per treatment and per control group were used. There were 36 adults and nauplii per replicate.			

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Exposure Route, Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Media. Path: Taxa, Species, Age: Invertebrate; Arthropods; Parvocalanus crassirostris; Not Applicable (e.g., fungi or algae studies) or Not Reported **Health Outcome:** Other (please specify below) (Population Size) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 3859142 Domain Metric Rating Comments Metric 16: Adequacy of Test Conditions Low Authors did not report environmental water quality conditions throughout the 24 day study. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology compared relative population size (+/- SE) to control replicate population size. Metric 18: Consistency of Outcome High Assessment within this multigenerational experiment appeared to be consistent among treatment and control groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low Environmental conditions were not reported to indicate if they caused any differences. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment.

Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors used ANOVA and Tukey's post hoc to test for differences among treatment and control groups. Metric 22: Reporting of Data High Section 3.3 and Figure 4 detail 6 day exposure to DEHP effects on the population size (page 5/8). Relative Parvocalanus crassirostris population size (mean \pm SE, n=4) was provided for each treatment group and control in Fig 4. Metric 23: Explanation of Unexpected Outcomes High Measures of variability in generation size relative to the control population are reported in section 3.3 as standard error (SE).

Additional Comments: DEHP exposures for 6 days and for 24 days significantly reduced the average population sizes (59±4.9% and 59±3.4%) relative to the control.

Overall Quality Determination

Study Citation:	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate			
	microparticle	es and Di(2-ethylhexyl)phthalate on the cala	noid copepod, I	Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10) days	
Exposure Route,	Aquatic (ma	rine); Water, Food/Diet; Dietary		
Media, Path:				
Taxa, Species, Age:	Invertebrate;	Arthropods; Parvocalanus crassirostris; Ad	lult	
Health Outcome:	Reproductive	e/Teratogenic		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	3859142	-		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is $>98\%$.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls are present and detailed in table 2A and 2B for this experiment.
	Metric 5:	Negative Control Response	High	Control eggs per female are reported in section 3.2 and in Figure 3 (page 5/8).
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Concentrations of the test substance were not measured during the study. For the 5 day exposure, the treatment solutions were renewed at day 3. Re- newal at day 3 is longer than the 24 hr renewal performed in the same paper for the acute bioassay.
	Metric 8:	Consistency of Exposure	High	There were 3 treatment concentrations, and they appeared to be consistently applied.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentra- tions.
	Metric 10:	Exposure Duration and Frequency	High	The study was over 5 days to allow for the zooplankton to grow eggs for reproductive output.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The acute bioassay used 3 concentrations with a control.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. The highest concentration used in the reproductive bioassay was 3.0 ng/L. The final Scope for DEHP lists the solubility for this compound as 2700 ug/L.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The reproductive experiment was conducted with inhouse copepod cultures.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups.
		Contin	nued on next pa	age

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Exposure Route, Aquatic (marine); Water, Food/Diet; Dietary Media. Path: Taxa, Species, Age: Invertebrate; Arthropods; Parvocalanus crassirostris; Adult **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 3859142 Domain Metric Rating Comments Number of Organisms and Metric 15: Medium The authors report 10 replicates per treatment concentration and 20 females per repli-Replicates per Group cate. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Authors did not report environmental water quality conditions throughout the 5 day study. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology resulted in mean (+/- SE) for eggs/female for the control and for three treatment concentrations. Metric 18: Consistency of Outcome High The assessment within this reproduction experiment appeared to be consistent among Assessment treatment and control groups. Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: Low Environmental conditions were not reported to indicate if they caused any differences. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors used ANOVA and Tukey's post hoc to test for differences among treatment and control groups. Metric 22: Reporting of Data High Section 3.2 details DEHP effects on the number of eggs per female, and Figure 3b presents the data for each treatment group and control (Page 5/8). Metric 23: Explanation of Unexpected Outcomes High Measures of variability in mean eggs per female are reported in section 3.2 as standard

Additional Comments: The concentration of DEHP was not measured, and environmental conditions during the 5 day exposure were not reported.

Overall Quality Determination

Medium

error (SE).

Study Citation:	Heindler, F.	M., Alajmi, F., Huerlimann, R., Zeng, C., Ne	wman, S. J., Var	nvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate			
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (ma	ation: > 21 days; Exposure Duration: > 21 rine); Water; Not determined by study authors	days days (i.e., chemica	al of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate	; Arthropods; Parvocalanus crassirostris; No	ot Applicable (e	.g., fungi or algae studies) or Not Reported			
Health Outcome:	Other (please specify below) (Population Size)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3859142						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical name was provided with correct nomenclature.			
	Metric 2:	Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.			
	Metric 3:	Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is $>98\%$.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Controls are present and detailed in section 2.4 and in Figure 1.			
	Metric 5:	Negative Control Response	High	Control population size after 24 days is presented in Figure 4. All treatment groups are compared as their % relative to the control population size at the end of the experiment.			
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the concentrations of the test substance were not measured during the study.			
	Metric 8:	Consistency of Exposure Administration	High	This study spanned over multiple generations (24 days) and only used one treatment concentration (0.11 ng/L), which was 10% of the 48hr LC50 for nauplii.			
	Metric 9:	Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentra- tions.			
	Metric 10:	Exposure Duration and Frequency	High	The study was 24 days to allow for the zooplankton to produce multiple generations.			
	Metric 11:	Number of Exposure Groups/	N/A	One treatment concentration (0.11 ng/L) was chosen to represent toxicity to 10% of the 48hr LC50 for nauplii			
	Metric 12:	Testing at or Below Solubility Limit	High	0.11 ng/L is under solubility limits. The Final Scope for DEHP lists the solubility for this compound as 2700 ug/L			
Domain 4: Test Organis	m						
U	Metric 13:	Test Organism Characteristics	High	The multigeneration experiment was conducted with inhouse copepod cultures.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
		Contin	nued on next pa	age			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page				
Study Citation:	Heindler, F. microparticl	M., Alajmi, F., Huerlimann, R., Zeng, C., Nev es and Di(2-ethylhexyl)phthalate on the cala	wman, S. J., Van noid copepod. F	nvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate Parvocalanus crassirostris. Ecotoxicology and Environmental Safety 141:298-305.				
Duration:	Overall Dura	all Duration: > 21 days; Exposure Duration: > 21 days tic (marine): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study autho	rs (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate	; Arthropods; Parvocalanus crassirostris; No	ot Applicable (e.	g., fungi or algae studies) or Not Reported				
Health Outcome:	Other (pleas	e specify below) (Population Size)						
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	3859142							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates per treatment group and control group were used. There were 36 adults and 68 nauplii per replicate.				
Domain 5: Outcome As	Ssessment	A de sus est of Trest Constitions	τ					
	Metric 16:	Adequacy of fest Conditions	Low	Authors did not report environmental water quality conditions throughout the 24 day study.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported relative population size (+/- SE) com- pared to control replicates.				
	Metric 18:	Consistency of Outcome	High	Assessment within this multigenerational experiment appeared to be consistent among				
		Assessment		treatment and control groups.				
Domain 6: Confoundin	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	Low	Environmental conditions were not reported to indicate if they caused any differences.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced outcome assessment.				
Domain 7: Data Brasan	tation and Anal	lucio						
Domain 7. Data Fresen	Matria 21	Statistical Matheda	High	Authors used ANOVA and Tukay's next has to test for differences among treatment and				
	Metric 21:	Statistical Methods	nigii	control groups.				
	Metric 22:	Reporting of Data	High	Section 3.3 and Figure 4 detail 24 day exposure to DEHP effects on the population size (page 5/8). Relative Parvocalanus crassirostris population size (mean \pm SE, n=4) were provided for each treatment group and control in Fig 4.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability in generation size relative to the control population are reported in section 3.3 as standard error (SE).				

Additional Comments: DEHP exposures for 6 and for 24 days significantly reduced the average population sizes (59±4.9% and 59±3.4%) relative to the control.

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Hobson, J. F	, Carter, D. E., Lightner, D. V. (1984). Toxi	city of a phthalate ester in	the diet of a penaied shrimp. Journal of Toxicology and Environmental
Duration:	Health 13(4- Overall Dura	·6):959-968. ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-96h)	
Exposure Route,	Aquatic (ma	rine); Food/Diet; Dietary		
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate ADME (bio Di-ethylhexy	Arthropods; <i>Penaeus vannamei</i> ; Not Applic transformation) yl phthalate (DEHP)	cable (e.g., fungi or algae	studies) or Not Reported
HERO ID:	679685			
Domain	<u></u>	Metric	Rating	Comments
Domain 1. Test Substan	Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name and form. Radiolabeled DEHP was synthesized from carbonyl-labeled 1,2- dicarboxylic acid (o-phthallic acid) and 2-ethylhexanol and purified by thin-layer chromatography.
	Metric 2:	Test Substance Source	Low	Radiolabeled DEHP was obtained from an individual, but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	Study authors reported using a concurrent control group. But the control was an unoccu- pied exposure chamber (no test animal) that received 600 ppm diet (dosed with DEHP) at each feeding. This was more of an analytical control to ensure that the DEHP was primarily found in the feed (Table 2). A true negative control was not included in the experiment.
	Metric 5:	Negative Control Response	Uninformative	There was no test animal placed in the control exposure chamber. Therefore, the biologi- cal response of the control could not be reported.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	Methods of dosing diets with radiolabeled DEHP were not provided, but the specific activities of the treatment groups at the beginning of the experiment were reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be ad- ministered consistently across study groups. It was reported that shrimp were fed at a rate of 1% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured. Specific activity of the feed was measured at the beginning and at the end of the experiment. Water samples were measured by scintillation counting at the beginning, prior to each feeding, and at the end of the experiment.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (24 and 96 hrs) was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.

Diethylhexyl Phthalate

HERO ID: 679685 Table: 1 of 1

		сог	ntinued from previous	page			
Study Citation:	Hobson, J. F Health 13(4-	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental Health 13(4-6):059.968					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-96h)				
Exposure Route,	Aquatic (ma	Aquatic (marine); Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Penaeus vannamei; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	679685						
Domain		Metric Rating Comments					
Domain 4: Test Organis	sm						
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized and/or whether pre-			
	Metric 15.	Number of Organisms and	Medium	1 organism per exposure chamber and 5 replicates per concentration were used			
	Weule 15.	Replicates per Group	Wiedium	i organism per exposure enamoer and 5 repredicts per concentration were used.			
		· · · · · · · · · · · · · · · · · · ·					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 24 hr and 96 hr uptake studies were not reported.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcomes of interest. Shrimp samples were oxidized using a a Packard B306 tissue oxidizer and analyzed by scintillation counting.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was conducted after 24 and 96 hours of exposure.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	ANOVA was performed to assess time and dose effects.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 3).			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	A true negati	ive control was not included in the experiment	t. Methods of dosing di	ets with DEHP were not provided.			

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Hobson, J. F Health 13(4-	F., Carter, D. E., Lightner, D. V. (1984). To -6):959-968.	oxicity of a phthala	te ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental
Duration: Exposure Route, Media Path:	Aquatic (ma	ation: 11 - 21 days; Exposure Duration: 1 urine); Food/Diet; Dietary	1 - 21 days	
Taxa, Species, Age: Health Outcome:	Invertebrate: Mortality	; Arthropods; Penaeus vannamei; Not App	plicable (e.g., fungi	or algae studies) or Not Reported
Chemical: HERO ID:	Di-ethylhex 679685	yl phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce		Ŧ	
	Metric 1:	Test Substance Identity	Low	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treat- ment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEPH. The control group, it seems, was fed the same diet, absent the test substance.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	Methods of dosing diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not, or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in the feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from the nominal concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be ad- ministered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors were derived from measured concentrations (Fig 2).
	Metric 10: Metric 11:	Exposure Duration and Frequency Number of Exposure Groups/ Spacing of Exposure Levels	High High	This was a 14 day dietary exposure to DEHP. The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study, which was to determine whether DEHP played a role in observed mortality rates in an aquaculture facility.

Continued on next page ...

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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 679685 Table: 1 of 3

		contin	ued from previ	ous page			
Study Citation:	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.						
Duration:	Overall Dur	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (ma	Aquatic (marine); Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Penaeus vannamei; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	679685						
Domain		Metric	Rating	Comments			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.			
Demain 4. Test Oreania							
Domain 4: Test Organis	Matria 12.	Test Organism Characteristics	Madium				
	Metric 15:	A colimatization and Protrootmont	Low	The study did not reported, but details of the test organism characteristics were facking.			
	Meuric 14.	Conditions	LOW	treatment conditions were the same for control and exposed groups			
	Metric 15:	Number of Organisms and	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.			
		Replicates per Group					
Domain 5: Outcome As	sessment		-				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in potential aqueous exposure.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment was conducted after 14 days of exposure.			
		Assessment	_				
Domain 6: Confounding	y / Variable Co	ntrol					
or comounding	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to the exposure.			
Domain /: Data Present	Auton and Anal	IVSIS Statistical Matheda	NT/A	Charlistical and have not measure for the measure literator. There is a final			
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for the mortality data. There were negative find- ings, even at the highest concentration tested.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	The method	s of dosing diets with DEHP were not provi	ided. Housing a	nd environmental conditions during the 14 day test were not reported. Measured			

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 679685 Table: 2 of 3

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Study Citation:	Hobson, J. F Health 13(4-	C., Carter, D. E., Lightner, D. V. (1984). To 6):959-968.	oxicity of a ph	thalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental				
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (ma	erall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days atic (marine); Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; ADME (biot Di-ethylhexy 679685	Arthropods; <i>Penaeus vannamei</i> ; Not App gransformation) yl phthalate (DEHP)	licable (e.g., t	fungi or algae studies) or Not Reported				
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].				
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.				
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treat-				
				ment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEPH. The control group, it seems, was fed the same diet, absent of the test substance.				
	Metric 5:	Negative Control Response	Medium	The biological responses of the negative control group was reported, but there was DEHP contamination in the control (2 ppm vs 44-50,227 ppm in the treatment groups).				
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Domain of Disposito on	Metric 7:	Experimental System/Test Media Preparation	Low	The methods of dosing the diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from nominal concentrations.				
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported and exposures appear to be admin- istered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feed- ing/exposure for all test organisms were not provided.				
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors derived from the measured concentrations (Fig 2).				
	Metric 10:	Exposure Duration and Frequency	High	This was a 14 day dietary exposure to DEHP.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.				

Domain 4: Test Organism

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Environmental Hazard Evaluation

HERO ID: 679685 Table: 2 of 3

		conti	nued from p	revious page	
Study Citation:	Hobson, J. F Health 13(4-	., Carter, D. E., Lightner, D. V. (1984). To: 6):959-968.	kicity of a ph	thalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental	
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days		
Exposure Route,	Aquatic (ma	rine); Food/Diet; Dietary	•		
Media, Path:	•	•			
Taxa, Species, Age:	Invertebrate;	Arthropods; Penaeus vannamei; Not Appl	icable (e.g.,	fungi or algae studies) or Not Reported	
Health Outcome:	ADME (biot	ADME (biotransformation)			
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	679685				
Domain		Metric	Rating	Comments	
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.	
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized and/or whether pre-	
		Conditions		treatment conditions were the same for control and exposed groups.	
	Metric 15:	Number of Organisms and	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in aqueous exposure.	
	Metric 17:	Outcome Assessment Methodology	Medium	Detailed methods for determining DEHP body burden were not provided.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted after 14 days of exposure.	
Domain 6: Confounding	r / Variable Cou	ntrol			
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental	
		Design and Procedures	2011	conditions or other non-treatment-related factors across study groups.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to the exposure.	
Domain 7: Data Present	ation and Anal	vsis			
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability for whole-body residues of DEHP for all treatment groups.	
Additional Comments:	Methods of preparing diets with DEHP were not provided. Housing and environmental conditions during the 14 day test were not reported. Measu concentrations were different from nominal concentrations. There was DEHP contamination in the control group. The study did not report measures variability for whole-body residues of DEHP for all treatment groups.			ng and environmental conditions during the 14 day test were not reported. Measured was DEHP contamination in the control group. The study did not report measures of bs.	
Overall Oualit	ty Detern	nination	Low		

Study Citation:	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Aquatic (marine); Food/Diet; Dietary
Media, Path:	
Taxa, Species, Age:	Invertebrate; Arthropods; Penaeus vannamei; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	679685

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treat- ment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEPH. The control group, it seems, was fed the same diet, absent of the test substance.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The methods of dosing diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not, or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in the feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from nominal concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be ad- ministered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors were derived from the measured concentrations (Fig 2).
	Metric 10:	Exposure Duration and Frequency	High	This was a 14 day dietary exposure to DEHP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.

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Environmental Hazard Evaluation

HERO ID: 679685 Table: 3 of 3

		contin	ued from previ	ous page			
Study Citation:	Hobson, J. F Health 13(4-	., Carter, D. E., Lightner, D. V. (1984). Toxi 6):959-968	city of a phthala	ate ester in the diet of a penaied shrimp. Journal of Toxicology and Environmental			
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days				
Exposure Route,	Aquatic (ma	rine); Food/Diet; Dietary					
Media, Path:	1						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Penaeus vannamei; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	679685						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in potential aqueous exposure.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted after 14 days of exposure.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to the exposure.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for development/growth data (molting).			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented in the results section in the text as a general assessment.			
	Metric 23.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (marine); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Non-vascular Plants; <i>Karenia brevis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP) 3230225						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	Low	Relevant test materials are described as di-iso-butyl ortho-phthalate (DIBP), benzyl-n-butyl ortho-phthalate (BBP), di-n-butylortho-phthalate (DBP), and bis(2- ethylhexyl)ortho-phthalate(DEHP). No further details are provided.			
	Metric 2:	Test Substance Source	Low	Chemicals are sourced by the Sigma Company; no additional information is provided.			
	Metric 3:	Test Substance Purity	High	The purity is reported as $> 99\%$.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Negative and solvent controls were used. The authors indicated that no significant differ- ences were observed between the solvent control (acetone concentration (0.5 mL L-1)) and the negative control.			
	Metric 5:	Negative Control Response	High	No adverse effects were reported in the control.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Cha	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and minimize loss of the test substance before and during the ex-			
	Metric 8:	Consistency of Exposure Administration	Low	posure for these degradable substances. A solvent (acetone) was used to facilitate the preparation of the stock solution. Reporting omissions are likely to have a substantial impact on results. No measurement of test material concentration was conducted at the end of the test, so the actual exposure concentration was uncertain. Similarly, the different biodegradation rates of the chemicals meant that exposure concentrations may have differed because of degradation, but this was not discussed.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported. As degradation was likely to be observed, the reported nominal concentrations were not likely to be representative of the final concentration, and reporting in terms of nominal concentrations may have underestimated the effects observed.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (96-hour algae growth inhibition test).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Exposure concentrations were reported to be 0, 1, 5, 10, 20, 30, 50, 100, 150, 200 mL/L. These concentrations of DEHP did not inhibit algal growth.			
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate (the authors demonstrated that no significant difference ($p > 0.05$) was observed between the growth in controls and acetone treatment.).			

Diethylhexyl Phthalate

		contin	nued from p	revious page			
Study Citation:Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-50Duration:Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)Exposure Route,Aquatic (marine); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptMedia, Path:route)Taxa, Species, Age:Vegetation; Non-vascular Plants; Karenia brevis; Not Applicable (e.g., fungi or algae studies) or Not ReportedHealth Outcome:Development/GrowthChemical:Di-ethylhexyl phthalate (DEHP)HERO ID:3230225							
Domain		Metric	Rating	Comments			
Domain 4: Test Organisi	m						
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized or whether pretreat- ment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms (or algal density) were not reported in each treatment group and control.			
Domain 5: Outcome Ass	recement						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	The algal cells were cultured in a GXZ-380Z intelligent illumination incubator under the following conditions: 14 h of light at 24C ($4000 \pm 500 \text{ lux}$) and 10 h of dark at 22C. The medium was shaken once daily to prevent cell adherence growth.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported; it was unclear whether methods were sensitive for the outcome of interest. The authors reported that no signif- icant effects were observed on growth following exposure to DEHP, but no data were provided			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistent across groups. Cell numbers were determined every 24 hours.			
Domain 6: Confounding	/ Variable Co	atrol					
Domain of Companying	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Presenta	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	One-way ANOVA was adopted to determine the significant differences between ex- perimental and control groups. The calculations and measures of significance were not provided, so no conclusions about a dose response could be made.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.			
Additional Comments:	There was no and DBP wa	o significant effect on algal growth followin s lacking.	ng DEHP ex	posure. The discussion of growth inhibition following exposure to DEHP, DIBP, BBP			

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Diethylhexyl Phthalate

		continued from previous page	·			
Study Citation:	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z.,	Zheng, H. (2016). Inhibitory mecha	nism of phthalate esters on Karenia brevis. Chemosphere 155:498-508.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	re Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (marine); Cell Culture Media; Not d	etermined by study authors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake			
Media, Path:	route)					
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Karenia brevis; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Development/Growth					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	3230225					
Domain	Metric	Rating	Comments			

Low

Study Citation:	Wofford, H.	W., Wilsey, C. D., Neff, G. S., Giam, C. S., I	Neff, J. M. (1981). Bioacc	cumulation and metabolism of phthalate esters by oysters, brown shrimp,
Duration: Exposure Route, Media. Path:	and sheepshe Overall Dura Aquatic (bra	ead minnows. Ecotoxicology and Environmentation: 0 - 4 days (0-96h); Exposure Duration ckish); Water; Not determined by study auth	ental Safety 5(2):202-210 : 0 - 4 days (0-96h) nors (i.e., chemical of inte	rest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	ish; Cyprinodon variegatus; Not Applicable	e (e.g., fungi or algae stud	ies) or Not Reported
Health Outcome:	ADME (biot	ransformation)		
Chemical: HERO ID:	Di-ethylhexy 789995	l phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were reported.
	Metric 2:	Test Substance Source	High	The test substance source includes: Aldrich Chemical Company for the unlabeled phtha- late and California Bionuclear Corporation for the 14C-labeled phthalate.
	Metric 3:	Test Substance Purity	Low	The purity of the chemical was not included in the study.
Domain 2: Test Design				
e	Metric 4:	Negative Controls	Uninformative	No negative controls were reported.
	Metric 5:	Negative Control Response	N/A	No negative controls were reported.
	Metric 6:	Randomized Allocation	Medium	Organisms were collected from Galveston Bay. The allocation method to exposure groups was not reported.
Domain 3: Exposure Ch	aracterization			
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for the test preparation was described. Measures taken to ac- count for P-chem properties were not reported.
	Metric 8:	Consistency of Exposure	High	No variations in exposure administration were reported.
	Metric 9:	Administration Measurement of Test Substance	High	Concentrations were measured using analytical techniques, gas-liquid chromatography and liquid scintillation
	Metric 10:	Exposure Duration and Frequency	Medium	There was a 24-hour exposure period.
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.
Domain 4: Test Organiza	m			
Domain 4. Test Organisi	Metric 13.	Test Organism Characteristics	Low	Organisms characteristics were not described in the study
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were acclimatized for 4 days prior to phthalate exposure
	Metric 15:	Conditions Number of Organisms and	Low	There was a lower than typical number of organisms in the exposure groups.
		Replicates per Group		
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
		С	continued on next page .	

Diethylhexyl Phthalate

		con	tinued from previous	page		
Study Citation:	Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.					
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration: () - 4 days (0-96h)			
Exposure Route,	Aquatic (brac	ckish); Water; Not determined by study author	s (i.e., chemical of inte	erest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	ish; Cyprinodon variegatus; Not Applicable (e.g., fungi or algae stud	lies) or Not Reported		
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	789995					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was addressed.		
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent for all groups.		
		Assessment				
Domain 6: Confounding	/ Variable Con	itrol				
-	Metric 19:	Confounding Variables in Test	High	No confounding variables were indicated in the assessment.		
		Design and Procedures	c	-		
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.		
Domain 7: Data Present	ation and Analy	ysis				
	Metric 21:	Statistical Methods	High	A three-way analysis of variance (ANOVA) on the data was performed using the Gen- eral Linear Model procedure of SAS 76.		
	Metric 22:	Reporting of Data	High	Data for each outcome was reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Overall Dura Aquatic (ma uptake route Vertebrate; F Mortality Di-ethylhexy 1316224	Springborn (1984). Acute toxicity of thirta ation: 0 - 4 days (0-96h); Exposure Duratio rine), Aquatic (brackish); Water; Not dete) Fish; <i>sheepshead minnow (Cyprinodon var</i> yl phthalate (DEHP)	een phthalate on: 0 - 4 days rmined by stu <i>iegatus)</i> ; Juve	esters to the sheepshead minnow (Cyprinodon variegatus) (final report). (0-96h) idy authors (i.e., chemical of interest in exposure water, but unable to determine exact enile
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	High High Low	The test material was identified, and the CASRN was given. The source was listed as EG&G Bionomics Aquatic Toxicology Laboratory in Ware- ham, Massachusetts. No other information about the source was given. The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4: Metric 5: Metric 6:	Negative Controls Negative Control Response Randomized Allocation	High High Medium	A negative control was used. No mortality was reported in the controls. Test organisms were impartially distributed to each chamber (pdf pg 136).
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail and accounted for the properties of the test material. For all low-solubility phthalates, an enhanced mixing procedure was used, while for the butyl benzyl phthalate exposure, microbial degradation was accounted for with a cleaning pro- cedure that was implemented daily. The authors reported significant degradation of the test material throughout the test, but quantified the degradation throughout the test and reported the endpoint in terms of mean-measured concentration, so this does not have an impact on the results.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using GS-MS to account for poor water solubility.
	Metric 10: Metric 11:	Exposure Duration and Frequency Number of Exposure Groups/ Spacing of Exposure Levels	Hıgh N/A	A 96 hour exposure was appropriate for an acute test. Test concentration spacing was limited, as this was designed as a limit test up to the solubility limit of the chemical.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit, which were reported in Appendix A (pdf pg 164).
Domain 4: Test Organisi	m			
	Metric 13:	Test Organism Characteristics	High	Specimens were either cultured at the Laboratory orpurchased commercially. All fish were tested as juveniles, <10weeks old.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	A 96 hour acclimation period was reported.
		Cont	inued on nex	t page

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Diethylhexyl Phthalate

HERO ID: 1316224 Table: 1 of 1

		conti	nued from p	revious page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	ly Citation:Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report).ration:Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)osure Route,Aquatic (marine), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exalia, Path:uptake route)a, Species, Age:Vertebrate; Fish; sheepshead minnow (Cyprinodon variegatus); Juveniledth Outcome:Mortalitygenical:Di-ethylhexyl phthalate (DEHP)RO ID:1316224								
Domain		Metric	Rating	Comments					
	Metric 15:	Number of Organisms and Replicates per Group	Low	Only two replicates of 10 fish were used in each treatment.					
Domain 5: Outcome A	ssessment								
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and biomass loading was appropriate.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes(s) of interest (mortality).					
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.					
Domain 6: Confoundir	og / Variable Cou	atrol							
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The study reported minor differences among the study groups with respect to environ- mental conditions or other non-treatment-related factors, but these are unlikely to have a substantial impact on results. Authors reported that dissolved oxygen fell below guide- line recommended levels, and control mortality was high for one phthalate (not this phthalate). As no mortalities were observed in any test concentrations for this chemical, this was not determined to affect this test.					
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure (e.g., infec- tion) were reported for each study group, and there were no differences among groups that could influence the outcome assessment.					
Domain 7: Data Preser	ntation and Anal	vsis							
_ 5111111 , , Dutu 1 10501	Metric 21:	Statistical Methods	N/A	No mortality was reported, so no statistical analysis was needed.					
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported quantitatively.					
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily ex- plained. Low DO did not have an affect on the outcome, and high mortality in controls was not reported for this chemical.					

1C, butyl benzyl phthalate was referred to as 1D, DIDP was referred to as 1L, and DINP was referred to as 1J.

Overall Quality Determination	High	

Study Citation:	Sugawara, N	. (1974). Toxic effect of a normal series o	f phthalate es	ters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-
Duration: Exposure Route, Media. Path:	89. Overall Dura Aquatic (bra	ation: 0 - 4 days (0-96h); Exposure Duration ckish); Water; Not determined by study au	on: 0 - 4 days uthors (i.e., ch	(0-96h) emical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Artemia salina; Embryo		
Health Outcome:	Mortality			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	1315792			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	Medium	Hatch rate of ~47% in the control seems low.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations, Triton X-100 used as a carrier. The experimental set up and the type of experimental vessel used were not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	A one-time dose was used. The volume of test solution in experimental dishes was not reported.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The text states 40 or 72 hours was the exposure duration. Figure 2 shows the number of eggs hatched after 40 hours. DOP is shown in this figure.
	Metric 11:	Number of Exposure Groups/	High	Three treatment levels with adequate spacing were used.
		Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	Medium	Since the phthalates used in this study were not completely soluble, 20uL of Triton X- 100 was added and vigorously shaken. The solvent concentration seemed high. "The concentration of Triton X-100 in the control, 10 ppm, and 20-ppm solutions was adjusted to 10 ppm by adding this reagent."
Domain 4. Test Organis	m			
Domain 4. 16st Organisi	Metric 13:	Test Organism Characteristics	Low	The source of the test eggs was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Low	One to 2 mg of eggs were placed in the dishes (seems like a wide range and no repli- cates were reported).

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 1315792 Table: 1 of 1

		conti	nueu moni p	revious page		
Study Citation:	Sugawara, N. (1974). Toxic effect of a normal series of phthalate esters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-					
Duration: Exposure Route, Media. Path:	89. Overall Dura Aquatic (bra	ation: 0 - 4 days (0-96h); Exposure Duratio ckish); Water; Not determined by study aut	n: 0 - 4 days thors (i.e., ch	(0-96h) emical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 1315792	Arthropods; <i>Artemia salina</i> ; Embryo yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 5: Outcome Ass	sessment					
	Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	Low Low Low	Environmental conditions were not sufficiently reported to evaluate if adequate. The outcome assessment methodology was not clearly reported. Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. The Fig 1 caption states that student's t test was used.		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group in Figure 2. The legend for the graph is not included. A general overview of the results was described in the text, but not many details were provided.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.		
Additional Comments:	This evaluati chemical inv	ion is for mortality assessment after exposi rentory form. Mortality of shrimp eggs in the	ure to DOP. I	DOP is the same chemical compound as DEHP, hence why DEHP was chosen for the 47%		

Study Citation: Duration:	Wofford, H. and sheepshe Overall Dura	W., Wilsey, C. D., Neff, G. S., Giam, C. S., Nead minnows. Ecotoxicology and Environmention: 0 - 4 days (0-96h); Exposure Duration:	Neff, J. M. (1981). Bioacc ental Safety 5(2):202-210 : 0 - 4 days (0-96h)	cumulation and metabolism of phthalate esters by oysters, brown shrimp,					
Exposure Route, Media. Path:	Aquatic (bra	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; ADME (biot Di-ethylhexy 789995	Mollusks; <i>Crassostrea virginica</i> ; Not Applic ransformation) /l phthalate (DEHP)	cable (e.g., fungi or algae	e studies) or Not Reported					
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.					
	Metric 2:	Test Substance Source	High	The test substance source includes: Aldrich Chemical Company for the unlabeled phtha- late and California Bionuclear Corporation for the 14C-labeled phthalate.					
	Metric 3:	Test Substance Purity	Low	The purity of the chemical was not included in the study.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	Uninformative	An experiment with control to test the absorbance of empty oyster shells was conducted. However, no controls were used for the experiment that measured concentrations in organism tissue samples.					
	Metric 5:	Negative Control Response	N/A	No negative controls were reported.					
	Metric 6:	Randomized Allocation	Low	Organisms were collected from Galveston Bay. The allocation method to the exposure groups was not reported.					
Domain 3: Exposure Ch	aracterization								
2 oniun et Enpoure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for the test preparation was described. Measures taken to ac- count for P-chem properties were not reported.					
	Metric 8:	Consistency of Exposure	High	No variations in the exposure administration were reported.					
	Metric 9:	Administration Measurement of Test Substance	High	Concentrations were measured using analytical techniques- gas-liquid chromatography					
	Metric 10	Concentration Exposure Duration and Frequency	Medium	This was a 21-hour exposure period					
	Metric 11	Number of Exposure Groups/	Low	Only two exposure groups were reported					
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.					
		~ ~							
Domain 4: Test Organis	m								
	Metric 13:	Test Organism Characteristics	Low	Organism characteristics were not described in the study.					
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were acclimatized for 4 days prior to phthalate exposure.					
	Metric 15:	Number of Organisms and Replicates per Group	Low	A lower than is typical number of organisms was used in the exposure groups.					
Diethylhexyl Phthalate

			indea from previoa	s hage				
Study Citation:	Wofford, H.	Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration: 0	- 4 days (0-96h)					
Exposure Route,	Aquatic (bra	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	•							
Taxa, Species, Age:	Invertebrate;	Mollusks; Crassostrea virginica; Not Applical	ble (e.g., fungi or alg	ae studies) or Not Reported				
Health Outcome:	ADME (biot	ADME (biotransformation)						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	789995	789995						
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology was addressed.				
	Metric 18:	Consistency of Outcome	High	The outcome assessment was consistent for all groups.				
		Assessment						
Domain 6: Confounding	g / Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were indicated in the assessment.				
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.				
Domain 7: Data Present	ation and Anal	veic						
Domain 7. Data Present	Metric 21:	Statistical Methods	High	A three-way analysis of variance (ANOVA) on the data was performed using the General Linear Model procedure of SAS 76.				
	Metric 22:	Reporting of Data	High	Data for each outcome was reported.				
	Metric 23:	Explanation of Unexpected Outcomes	Medium	The concentrations during the 24-hr exposure period remained relatively constant with the exception of the oyster experiments. The concentrations decreased 30 to 70% during the oyster exposures, probably due to the high adsorptive capacity of the shells. The assertion was supported by an experiment showing absorption of chemical concentration in empty ovster shells.				

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Forget-Leray copepod Eury Overall Dura Aquatic (brac Invertebrate; Mortality Di-ethylhexy 679508	, J., Landriau, I., Minier, C., Leboulenger, I ytemora affinis (Poppe). Ecotoxicology an tion: 4 - 10 days; Exposure Duration: 4 - 1 ekish); Water; Not determined by study au Arthropods; <i>Eurytemora affinis</i> ; Larvae l phthalate (DEHP)	F. (2005). Im d Environme l0 days thors (i.e., ch	pact of endocrine toxicants on survival, development, and reproduction of the estuarine ental Safety 60(3):288-294. nemical of interest in exposure water, but unable to determine exact uptake route)
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [di(ethyl-hexyl)-phthalate, DEHP].
	Metric 2:	Test Substance Source	Low	substance source information that was given was the manufacturer. All test substances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.Certain test substances required DMSO to be used as a carrier solvent at a maximum concentration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used the carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
	, . <i>,</i> .			
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately re- ported for each individual chemical tested. The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were there- fore an overestimation of actual contaminants present."
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured (nominal concentrations used).
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.

Diethylhexyl Phthalate

		contin	nued from p	revious page			
Study Citation:	Forget-Leray	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copered Eurotemental Safety 60(3):288, 294					
Duration	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route	Aquatic (bra	ckish): Water: Not determined by study aut	thors (i.e., ch	emical of interest in exposure water, but unable to determine exact untake route)			
Media, Path:	riquate (bra	Aquate (orackisii), water, ivot determined by study autions (i.e., chemical or interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Eurytemora affinis; Larvae					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	679508						
Domain		Metric	Rating	Comments			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.			
	Metric 15	Conditions Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient			
	Methe 15.	Replicates per Group	Wiedrum	to characterize toxicological effects.			
		Repleates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	/ Variable Co	ntrol					
······································	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.			
		Design and Procedures	U				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	veie					
Domain 7. Data Present	Metric 21	Statistical Methods	High	Statistical methods were clearly described			
	Metric 22:	Reporting of Data	Medium	Endpoints were reported but treatment values were not			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
		r a sheri e e e recente	8	· · · · · · · · · · · · · · · · · · ·			
Additional Comments:	This evaluati	ion is for the 96H LC50 value.					
Overall Qualit	y Detern	nination	High				

Study Citation:	Forget-Leray	, J., Landriau, I., Minier, C., Leboulenger, I	F. (2005). Im d Environme	pact of endocrine toxicants on survival, development, and reproduction of the estuarine ntal Safety 60(3):288-294
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (brac	tion: 4 - 10 days; Exposure Duration: 4 - 1 kish); Water; Not determined by study aut	0 days thors (i.e., ch	emical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 679508	Arthropods; <i>Eurytemora affinis</i> ; Larvae l phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The chemical was identified by the accepted name [di(ethyl-hexyl)-phthalate, DEHP]. The only test substance source information that was given was the manufacturer."All test substances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group Certain
	Metrie I.		mgn	test substances required DMSO to be used as a carrier solvent at a maximum concen- tration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Demain 2: Engenne Ch				
Domain 3: Exposure Cha	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately re- ported for each individual chemical tested. The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were there- fore an overestimation of actual contaminants present."
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured (nominal concentrations used).
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.
Domain 4: Test Organise	n			
Domain 4. 16st Organisi	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.
		Conti	nued on nex	t page

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Diethylhexyl Phthalate

		conti	nued from p	revious page				
Study Citation:	Forget-Leray copepod Eur	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod Eurytemora affinis (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.						
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	l0 days					
Exposure Route,	Aquatic (bra	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Eurytemora affinis; Larvae						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	679508							
Domain		Metric	Rating	Comments				
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Statistical methods were clearly described.				
	Metric 22:	Reporting of Data	Medium	Endpoints were reported but treatment values were not.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	This evaluati	on is for the 10 d LOEC and NOEC values	5.					
		• •						

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Forget-Leray copepod Eur Overall Dura Aquatic (bra Invertebrate;	7, J., Landriau, I., Minier, C., Leboulenger, ytemora affinis (Poppe). Ecotoxicology ar ation: > 21 days; Exposure Duration: > 2 ckish); Water; Not determined by study au Arthropods; <i>Eurytemora affinis</i> ; Larvae	F. (2005). Im ad Environme 1 days thors (i.e., ch	pact of endocrine toxicants on survival, development, and reproduction of the estuarine ntal Safety 60(3):288-294. emical of interest in exposure water, but unable to determine exact uptake route)
Health Outcome:	Developmen	t/Growth		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	679508			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce	Trat Cubatan an Idantita	II: -h	
	Metric 1: Matria 2:	Test Substance Identity	High	The entry test substance source information given is the manufacturer" All test sub-
	Metric 2.	Test Substance Source	Low	stances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group Certain
	Meure 4.	ivegative controls	mgn	test substances required DMSO to be used as a carrier solvent at a maximum concen- tration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used the carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately re- ported for each individual chemical test. The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were there- fore an overestimation of actual contaminants present."
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured (nominal concentrations used).
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was less than 28 days but probably close to 14 days based on graph depiction.
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.

Diethylhexyl Phthalate

		contir	ued from p	revious page				
Study Citation:	Forget-Leray copepod Eur	orget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine opepod Eurytemora affinis (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.						
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days					
Exposure Route,	Aquatic (bra	ckish); Water; Not determined by study aut	hors (i.e., ch	emical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	•							
Taxa, Species, Age:	Invertebrate;	vertebrate; Arthropods; Eurytemora affinis; Larvae						
Health Outcome:	Developmen	evelopment/Growth						
Chemical:	Di-ethylhexy	pi-ethylhexyl phthalate (DEHP)						
HERO ID:	679508	579508						
Domain		Metric	Rating	Comments				
Domain 4: Test Organis	n							
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.				
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.				
	Metric 15:	Conditions Number of Organisms and	Low	The number of test organisms were not reported.				
		Replicates per Group						
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
Domain 6: Confounding	/ Variable Cor	ntrol						
c	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.				
		Design and Procedures	U					
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.				
Domain 7. Data Present	ation and Anal	vsis						
D official / D usu 1105010	Metric 21	Statistical Methods	High	The statistical methods were described				
	Metric 22:	Reporting of Data	High	Developmental time was reported via graph				
	Metric 22:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes				
		Explanation of Chexpected Outcomes	mgn					
Additional Comments:	This evaluati	on is for the inhibition of larval developme	nt.					
Overall Qualit	y Detern	nination	High					

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Study Citation:	Bionomics,,	EG&G (1984). Acute toxicity of twelve pl	hthalate ester	s to mysid shrimp (Mysidopsis bahia).	
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	(0-96h)	
Exposure Route,	Aquatic (ma	rine), Aquatic (brackish); Water; Not dete	rmined by stu	dy authors (i.e., chemical of interest in exposure water, but unable to determine exact	
Media. Path:	uptake route)	2		
Taxa, Species, Age:	Invertebrate:	Arthropods: <i>Mysidopsis bahia</i> : Juvenile			
Health Outcome:	Mortality				
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	1316220	- p			
Domain	1010220	Matric	Dating	Comments	
Domain 1: Tast Substan	22	Wethe	Kating	Comments	
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No CASRN or structure were	
	Metric 2:	Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA)	
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the substance were not included in the study.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Authors reported using negative controls.	
	Metric 5:	Negative Control Response	High	The response of the negative controls was adequate.	
	Metric 6:	Randomized Allocation	Medium	Mysid shrimp were maintained 1-3 days before they were distributed into test vessels.	
				J	
Domain 3: Exposure Ch	aracterization				
ľ	Metric 7:	Experimental System/Test Media Preparation	High	The experimental design followed protocol guidelines.	
	Metric 8:	Consistency of Exposure	High	Authors reported consistent administration.	
	Metric 9:	Administration Measurement of Test Substance	High	Phthalates were analytically verified and measured.	
	Metric 10:	Exposure Duration and Frequency	High	The test duration followed protocol	
	Metric 11:	Number of Exposure Groups/	Low	This study only performed a single exposure (0.44 mg/L) as a range finding test re-	
		Spacing of Exposure Levels	2011	ported no effect at levels below the water solubility limit.	
	Metric 12:	Testing at or Below Solubility Limit	Low	The single concentration used for DEHP was 0.44 mg/L and is over the solubility listed in the Final Scope (0.27 mg/L).	
Domain 4: Test Organis	m				
8	Metric 13:	Test Organism Characteristics	Medium	The source of the organisms was reported. Details beyond that were not reported.	
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were housed for 1-3 days prior to treatment.	
		Conditions			
	Metric 15:	Number of Organisms and	Medium	Replicates followed protocol.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	High	Environmental conditions were adequate and described in detail	
	Metric 17	Outcome Assessment Methodology	High	Outcomes were reported and addressed	
		~		· · · · · · · · · · · · · · · · · · ·	
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Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Bionomics,,	EG&G (1984). Acute toxicity of twelve ph	nthalate ester	s to mysid shrimp (Mysidopsis bahia).			
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	on: 0 - 4 days	(0-96h)			
Exposure Route,	Aquatic (ma	Aquatic (marine), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Media, Path:	uptake route	iptake route)					
Taxa, Species, Age:	Invertebrate;	invertebrate; Arthropods; Mysidopsis bahia; Juvenile					
Health Outcome:	Mortality	Aortality					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1316220						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed and reported.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	No differences were reported.			
		Design and Procedures	0	I			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.			
Damain 7. Data Durant							
Domain /: Data Present	Allon and Anal		T				
	Metric 21:	Statistical Methods	Low	No data analysis was presented as the LC50 was >0.44 mg/L.			
	Metric 22:	Reporting of Data	High	Data was reported adequately.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	ditional Comments: Based on preliminary studies, concentrations of DEHP below the solubility limit did not result in adverse outcomes; therefore, a single concentration w included in the final study.						
Overall Quali	ty Detern	nination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Linden, E., 1 water organi Overall Dura Aquatic (bra Invertebrate; Mortality Di-ethylhexy 51937	Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Nitocra spinipes</i> ; Adult Mortality Di-ethylhexyl phthalate (DEHP) 51937				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported, so an assessment of the exposure methods was difficult.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure and the exposure frequency were reported and suitable, but they were slightly longer than typical for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	At least 6 concentrations were tested, but a range was not reported.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit. However, the given LC50 ($>300 \text{ mg/L}$) is well above the water solubility given in the Final Scope for DEHP, 0.27 mg/L at 25C. This suggests that no toxicity was observed at saturation.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	The source of the test animals was not reported.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was unclear if the test organisms were acclimatized to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 20 organisms with no replicates per treatment used.		
Domain 5: Outcome As	sessment					

Diethylhexyl Phthalate

		continu	ued from previ	ous page				
Study Citation:	Linden, E., I	Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish						
	water organis	water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Aquatic (brad	ckish); Water; Not determined by study auth-	ors (i.e., chemic	al of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Nitocra spinipes; Adult						
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	51937							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and seemed consistent.				
Domain 6: Confounding	/ Variable Cor	itrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.				
Domain 7: Data Present	ation and Analy	ysis						
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.				
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the table.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	The given LO suggests that	C50 is unbounded (>300 mg/L), which is we no toxicity was observed at saturation.	ell above the wa	ter solubility limit given in the Final Scope for DEHP (0.27 mg/L at 25C), which				

Overall Quality Determination	Medium
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Study Citation:	Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp,							
Duration: Exposure Route, Media. Path:	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Invertebrate;	ivertebrate; Arthropods; <i>Penaecus aztecus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Chemical: HERO ID:	Di-ethylhexy 789995	/l phthalate (DEHP)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.				
	Metric 2:	Test Substance Source	High	Test substance sources include Aldrich Chemical Company for the unlabeled phthalate and California Bionuclear Corporation for the 14C-labeled phthalate.				
	Metric 3:	Test Substance Purity	Low	The purity of the chemical were not included in the study.				
Domain 2: Test Design								
e	Metric 4:	Negative Controls	Uninformative	Negative controls were not reported in the study.				
	Metric 5:	Negative Control Response	N/A	Negative controls were not reported in the study.				
	Metric 6:	Randomized Allocation	Medium	Organisms were collected from Galveston Bay. The allocation method to the exposure groups was not reported.				
Damain 2. England Ch								
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Media	Madium	The eventimental design for the test monomation was described				
	Wietife 7.	Preparation	Medium	The experimental design for the test preparation was described.				
	Metric 8:	Consistency of Exposure	High	No variations in exposure administration were reported				
	methe o.	Administration	mgn	no variations in exposure administration were reported.				
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using analytical techniques including gas-liquid chro- matography and liquid scintillation.				
	Metric 10:	Exposure Duration and Frequency	Medium	This was a 24-hour exposure period.				
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.				
Damain 4: Tast O								
Domain 4: Test Organisi	III Metric 12:	Test Organism Characteristics	Low	Characteristics were not described in the study				
	Metric 14	Acclimatization and Pretreatment	Luw High	Organisms were acclimatized for 4 days prior to phthalate exposure				
	Metric 15	Conditions Number of Organisms and	Low	There was a lower than typical number of organisms in the exposure groups				
		Replicates per Group	20.1	a to to that of pour name of organisms in the opposite groups.				
Domain 5: Outcome Ass	sessment							
20main 9. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.				
		(Continued on next page .					

Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Wofford, H. and sheepshe	Wofford, H. W., Wilsey, C. D., Neff, G. S., Giam, C. S., Neff, J. M. (1981). Bioaccumulation and metabolism of phthalate esters by oysters, brown shrimp, and sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Penaecus aztecus; Not Applicable	e (e.g., fungi or algae	studies) or Not Reported		
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	789995					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The methodology was addressed.		
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent for all groups.		
		Assessment				
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	High	No confounding variables were indicated in the assessment.		
		Design and Procedures	C			
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	A three-way analysis of variance (ANOVA) on the data was performed using the Gen- eral Linear Model procedure of SAS 76.		
	Metric 22:	Reporting of Data	High	Data for each outcome was reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route,	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure of Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Reproductive Di-ethylhexy 3071101	Mammalian; <i>Bos taurus, Holstein Fresian</i> ; e/Teratogenic /l phthalate (DEHP)	Embryo		
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl) phthalate (DEHP) and a commer- cial name. Oxoplast Q 30, along with purity and impurity.	
	Metric 2:	Test Substance Source	High	The source was identified as ZAK Spolka Akcyjna, a major chemical manufacturer in Poland.	
	Metric 3:	Test Substance Purity	High	The test substance purity was 99.5%, with impurities less than or equal to 0.1% .	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative controls $(N = 5)$ were tube-fed with water.	
	Metric 5:	Negative Control Response	High	Controls responded as expected.	
	Metric 6:	Randomized Allocation	Low	The researchers did not report randomization.	
Domain 3 [,] Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was adequately detailed.	
	Metric 8:	Consistency of Exposure Administration	High	Test substance exposures were administered consistently across the control and study group.	
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations (100 mg/kg of body weight) were measured prior to adminis- tration via tube-feeding.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration (3 days) and frequency (daily) were appropriate for the outcomes measured.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Single exposure concentration versus the control may be appropriate for determining effects for the purpose of the study, but would not be useful for determining a dose-response relationship.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was administered via tube-feeding.	
Domain 4: Test Organisi	m				
	Metric 13:	Test Organism Characteristics	Medium	The study was conducted with female test species on an experimental dairy farm, but no other details (i.e. age, weight/size, etc.) were provided.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were adequate and consistent across the control and exposed test groups.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of controls $(N = 5)$ and exposure test organisms $(N = 4)$ were adequate for characterizing toxicological effects.	

Diethylhexyl Phthalate

		conti	nued from p	previous page		
Study Citation:	Kalo, D., Ha Ovarian Fun	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary				
Media, Path:		•				
Taxa, Species, Age:	Vertebrate; N	Mammalian; Bos taurus, Holstein Fresian; I	Embryo			
Health Outcome:	Reproductiv	e/Teratogenic	-			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	3071101					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were adequate for the test species.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology measured the intended outcomes of interest (in vitro fertilization and embryo maturation).		
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the control and exposure groups outside the test substance administration.		
	Metric 20:	Outcomes Unrelated to Exposure	High	The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.		
Domain 7: Data Present	Domain 7. Data Presentation and Analysis					
	Metric 21:	Statistical Methods	High	Data were arcsine-transformed prior to one-way ANOVA followed by the Tukey-Kramer test.		
	Metric 22:	Reporting of Data	High	Data on developmental effects was presented in Figures 7-9.		
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in oocyte developmental effects and embryo competence from test substance exposures.		
Additional Comments:	None			• * * *		

Overall Quality Determination

High

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Bos taurus, Holstein Fresian</i>; Adult Mechanistic-Endocrine toxicity-Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 3071101 				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl) phthalate (DEHP) and a commer- cial name, Oxoplast O 30, along with purity and impurity.	
	Metric 2:	Test Substance Source	High	The source was identified as ZAK Spolka Akcyjna, a major chemical manufacturer in Poland.	
	Metric 3:	Test Substance Purity	High	The test substance purity was 99.5%, with impurities less than or equal to 0.1%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative controls $(N = 5)$ were tube-fed with water.	
	Metric 5:	Negative Control Response	High	Controls responded as expected.	
	Metric 6:	Randomized Allocation	Low	The researchers did not report randomization.	
Domain 3: Exposure Ch	aracterization				
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was adequately detailed.	
	Metric 8:	Consistency of Exposure Administration	High	Test substance exposures were administered consistently across the control and study groups.	
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations (100 mg/kg of body weight) were measured prior to admin- istration via tube-feeding. Metabolites were measured from blood, urine and follicular fluid, and milk from both control and treated test organisms.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration (3 days) and frequency (daily) were appropriate for the outcomes measured.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Single exposure concentration versus the control may be appropriate for determining effects for the purpose of the study, but would not be useful for determining a dose-response relationship.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was administered via tube-feeding.	
Domain 4. Toot Ora	~				
Domain 4: Test Organisi	Metric 13:	Test Organism Characteristics	Medium	The study was conducted with female test species on an experimental dairy farm, but no other details (i.e. age, weight/size, etc.) were provided.	
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were adequate and consistent across the control and exposed test groups.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of controls (N = 5) and exposure test organisms (N = 4) were adequate for characterizing toxicological effects.	

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 3071101 Table: 2 of 3

		contir	nued from p	orevious page			
Study Citation:	Kalo, D., Ha Ovarian Fun	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: > 21 day	S			
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; N	Mammalian; Bos taurus, Holstein Fresian; A	Adult				
Health Outcome:	Mechanistic	-Endocrine toxicity-Reproductive/Teratoger	nic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3071101						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were adequate for the test species.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology measured the intended outcomes of interest (metabolites of DEHP).			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the control and exposure groups outside the test substance administration.			
	Metric 20:	Outcomes Unrelated to Exposure	High	The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	DEHP-metabolite mean concentrations were analyzed by one-way ANOVA followed by Student's t test.			
	Metric 22:	Reporting of Data	High	Data on developmental effects was presented in Table 2 and Figure 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in DEHP metabolite concen- trations from test substance exposures.			
Additional Comments:	This form is	to account for cellular and biochemical out	comes of fo	llicle development in the ovaries and the effect on progesterone in the plasma.			
Overall Quali	ty Detern	nination	High				

Study Citation:	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on						
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; F	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Vertebrate; Mammalian; Bos taurus, Holstein Fresian; Adult						
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3071101						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl) phthalate (DEHP) and a commer- cial name, Oxoplast O 30, along with purity and impurity.			
	Metric 2:	Test Substance Source	High	The source was identified as ZAK Spolka Akcyjna, a major chemical manufacturer in Poland.			
	Metric 3:	Test Substance Purity	High	The test substance purity was 99.5%, with impurities less than or equal to 0.1%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative controls $(N = 5)$ were tube-fed with water.			
	Metric 5:	Negative Control Response	High	Controls responded as expected.			
	Metric 6:	Randomized Allocation	Low	The researchers did not report randomization.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was adequately detailed.			
	Metric 8:	Consistency of Exposure Administration	High	Test substance exposures were administered consistently across the control and study group.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations (100 mg/kg of body weight) were measured prior to adminis- tration via tube-feeding.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration (3 days) and frequency (daily) were appropriate for the outcomes measured.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Single exposure concentration versus the control may be appropriate for determining effects for the purpose of the study, but would not be useful for determining a dose-response relationship.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was administered via tube-feeding.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Medium	The study was conducted with female test species on an experimental dairy farm, but no other details (i.e. age, weight/size, etc.) were provided.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were adequate and consistent across the control and exposed test groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of controls $(N = 5)$ and exposure test organisms $(N = 4)$ were adequate for characterizing toxicological effects.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were adequate for the test species.			
		Cont	tinued on nex	t page			

Diethylhexyl Phthalate

continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover Effects of Acute DEHP Exposure on Ovarian Function and Oocyte Developmental Competence in Lactating Cows. PLoS ONE 10(7):e0130896. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Bos taurus, Holstein Fresian</i>; Adult Development/Growth Di-ethylhexyl phthalate (DEHP) 3071101 				
Domain		Metric	Rating	Comments	
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology measured the intended outcomes of interest (follicular and corpus luteum development and hormone concentrations). Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.	
Domain 6: Confounding	g / Variable Co		TT: 1		
	Metric 19: Metric 20:	Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High	There were no reported differences among the control and exposure groups outside the test substance administration. The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Data were analyzed by one-way ANOVA followed by the Student's t-test.	
	Metric 22: Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in developmental effects from test substance exposures.	
Additional Comments:	None				
Overall Quality Determination High					

Study Citation: Duration: Exposure Route, Media, Path:	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Vertebrate; N	Vertebrate; Mammalian; Common marmosets (Callithrix jacchus); Adult					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	aling/function-C	Oxidative stress (including redox biology)			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	630680						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical is identified as di(2-ethylhexyl)phthalate (DEHP), but no other identifying information is provided.			
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.			
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.			
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	DEHP was dissolved in corn oil and administered orally with a catheter once daily in the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.			
	Metric 8:	Consistency of Exposure Administration	High	The exposure solution volume or the number of molecules of the test substance per container were the same across replicates and groups.			
	Metric 9:	Measurement of Test Substance	Low	Authors allude to verification of the test material concentration, but exposure concentra- tions were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Dosages of 2500, 500, and 100 mg/kg were chosen based on the results of range-finding tests conducted before the initiation of the definitive test.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure therefore solubility was not relevant as it was administered via gavage.			
Domain 4. Test Organis	m						
20main 1. Test Organis	Metric 13:	Test Organism Characteristics	Medium	Test organisms were sourced from "CLEA JAPAN" and were separated by sex. The age of the test organisms was not specified, which could have affected the outcome of the test.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Marmosets were acclimated for 3 months in the same conditions as the test.			
Continued on next page							

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

HERO ID: 630680 Table: 1 of 4

		····contin	lucu nom prev	ious page				
Study Citation:	Kurata, Y., marmosets:	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Vertebrate; N	Mammalian; Common marmosets (Callithri	<i>x jacchus)</i> ; Adul					
Chamical	Di athulhaya	-Biomarkers (exposure and effect)-Cell sign	aling/function-	Jaidative stress (including redox biology)				
HERO ID.	630680	(DEIII)						
	050000		р. /:					
Domain	Matria 15.	Metric	Rating	Comments				
	Metric 15:	Replicates per Group	Low	use of replication was unclearly described.				
				X V				
Domain 5: Outcome A	ssessment							
	Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for mar- mosets was maintained at a room temperature of $26 \pm 2^{\circ}$ C, a relative humidity of $50 \pm$ 10%, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 1 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and ad- ditives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given assupplemental food Tap water passed through a 5-/xm filter andirradiated by ultraviolet was freely available to the animalsfrom water bottles.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.				
Domain 6: Confoundin	og / Variable Co	ntrol						
Bomain 0. Combuildin	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental condition				
	Metric 17.	Design and Procedures	mgn	or other factors that could influence the outcome assessment. There are no limitations that would result in a substantial impact on results.				
	Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced dispropor- tionate test organism attrition or outcomes unrelated to exposure. Males were reported have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an in- fluence on the meanvalues of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathologicalchanges suggestive of tract im pairment were noted inthose animals.				

Diethylhexyl Phthalate

	cont	inued from previ	ious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chamingh	 Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i>; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology) 				
HERO ID:	630680				
Domain	Metric	Rating	Comments		
	Metric 21: Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group vari- ances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).		
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.		
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.		
Additional Comments:	The authors characterized the levels of several biomarkers and mechanistic effects of the test organisms. These included: hepatic peroxisomal enzyme activities (catalase, D-Amino acid oxidase, cyanide-insensitive acyl CoA oxidation system, camitine-dependent acetyltransferase, camitine-dependent palmitovltransferase), hepatic microsomal protein, and cytochrome p-450 content.				

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media Path:	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Vertebrate; M	Vertebrate; Mammalian; Common marmosets (Callithrix jacchus); Adult					
Health Outcome:	Endocrine						
Chemical: HERO ID:	D1-ethylhexy 630680	Di-ethylhexyl phthalate (DEHP)					
Domain	000000	Metric	Rating	Comments			
Domain 1: Test Substand	ce	incure	Runng	Comments			
	Metric 1:	Test Substance Identity	Low	The chemical is identified as di(2-ethylhexyl)phthalate (DEHP), but no other identifying information is provided.			
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.			
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.			
Domain 2: Tast Dasign							
Domain 2. Test Design	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.			
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2. Euroques Ch	anastanization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	DEHP was dissolved in corn oil and administered orally with a catheter once daily in the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.			
	Metric 8:	Consistency of Exposure Administration	High	The exposure solution volume or the number of molecules of the test substance per container were the same across replicates and groups.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Authors allude to verification of the test material concentration, but exposure concentra- tions were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Dosages of 2500, 500, and 100 mg/kg were chosen based on the results of range-finding tests conducted before the initiation of the definitive test.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was dietary exposure, so solubility was not relevant as it was administered via gavage.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Medium	Test organisms were sourced from "CLEA JAPAN" and were separated by sex. Age of the test organisms was not specified, which could have affected the outcome of the test.			
	Metric 14:	Acclimatization and Pretreatment	High	Marmosets were acclimated for 3 months in the same conditions as the test.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	20 males and 20 females were selected and divided intofive groups equal in size. The use of replication was unclearly described.			
				· · ·			

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Kurata, Y., I marmosets: I Overall Dura Terrestrial; F Vertebrate; M Endocrine Di-ethylhexy 630680	Kidachi, F., Yokoyama, M., Toyota, N., Ts Lack of hepatic peroxisome proliferation, ter- tion: > 21 days; Exposure Duration: > 21 d ood/Diet; Dietary Mammalian; <i>Common marmosets (Callithrix</i> 1 phthalate (DEHP)	suchitani, M., K sticular atrophy, days <i>: jacchus)</i> ; Adult	Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.
Domain		Metric	Rating	Comments
Domain 5: Outcome As	sessment	A dama an of Track Care ditions	II:-h	
	Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for mar- mosets was maintained at a room temperature of $26 \pm 2^{\circ}$ C, a relative humidity of $50 \pm$ 10%, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and ad- ditives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given assupplemental food. Tap water passed through a 5-/xm filter andirradiated by ultraviolet was freely available to the animalsfrom water bottles.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding	y / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. There are no limitations that would result in a substantial impact on results.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced dispropor- tionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an in-

Domain 7: Data Presentation and Analysis

Continued on next page ...

fluence on the meanvalues of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathological changes suggestive of tract im-

pairment were noted inthose animals.

Diethylhexyl Phthalate

		continu	ued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	 Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i>; Adult Endocrine Di-ethylhexyl phthalate (DEHP) 			
HERO ID:	630680			
Domain		Metric	Rating	Comments
	Metric 21:	Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group vari- ances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily ex- plained.
	The such and		4 41	

The authors reported effect on endocrine activity by characterizing the effects on the levels of blood testosterone, estradiol, and cholecystokinin in the test Additional Comments: organisms. They also examine organ weight of the testis

Overall Quality Determination

Medium

Study Citation:	Kurata, Y.,	Kidachi, F., Yokoyama, M., Toyota, N., Ta	suchitani, M., k	Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common		
	marmosets:	Lack of hepatic peroxisome proliferation, te	sticular atrophy,	or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; 1	Food/Diet; Dietary	-			
Media, Path:		•				
Taxa, Species, Age:	Vertebrate:	Mammalian: Common marmosets (Callithrix	<i>ciacchus</i>): Adul	t		
Health Outcome:	Other (pleas	se specify below) (Organ Weight)	j			
Chemical:	Di-ethylbex	vl phthalate (DEHP)				
HERO ID:	630680					
Domain		Matric	Dating	Comments		
Domain 1: Test Substan	CP.	Wettic	Katilig	Comments		
Domain 1. Test Substan	Matric 1:	Test Substance Identity	Low	The abamical is identified as di(2 athylhavyl) witholate (DEUD) but no other identifying		
	wieure 1.	Test Substance Identity	Low	information is provided.		
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.		
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.		
Domain 2: Test Design			TT: 1			
	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.		
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	High	DEHP was dissolved in corn oil and administered orally with a catheter once daily in		
		Preparation		the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.		
	Metric 8:	Consistency of Exposure Administration	High	The exposure solution volume or the number of molecules of the test substance per container were the same across replicates and groups.		
	Metric 9:	Measurement of Test Substance	Low	Authors allude to verification of the test material concentration, but exposure concentra-		
		Concentration		tions were not measured or measurements were not reported.		
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).		
	Metric 11:	Number of Exposure Groups/	High	Dosages of 2500, 500, 100 mg/kg were chosen based on the results of range-finding		
		Spacing of Exposure Levels		tests conducted before the initiation of the definitive test.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure, so solubility was not relevant as it was administered via		
				gavage.		
Domain 4: Test Organis	m					
Domain 4. Test Organis	Metric 12.	Test Organism Characteristics	Medium	Test organisms were sourced from "CI EA IADAN" and were concreted by say. The acc		
	Metric 15.	Test Organism Characteristics	Wedlulli	of the test organisms was not specified, which could have affected the outcome of the test		
	Metric 14:	Acclimatization and Pretreatment	High	Marmosets were acclimated for 3 months in the same conditions as the test.		
		Conditions	-			
	Metric 15:	Number of Organisms and	Low	20 males and 20 females were selected and divided intofive groups equal in size. The		
		Replicates per Group		use of replication was unclearly described.		
		Contin	nued on next pa	age		

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Diethylhexyl Phthalate

		contir	nued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i>; Adult Other (please specify below) (Organ Weight) Di-ethylhexyl phthalate (DEHP) 630680 				
Domain		Metric	Rating	Comments	
Domain 5: Outcome As	Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	High High High	Throughout the study including acclimatization, the exclusive rearing room for mar- mosets was maintained at a room temperature of $26 \pm 2^{\circ}$ C, a relative humidity of $50 \pm 10\%$, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and ad- ditives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given assupplemental food. Tap water passed through a 5-/xm filter andirradiated by ultraviolet was freely available to the animalsfrom water bottles. The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the out- comes(s) of interest. Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. There are no limitations that would result in a substantial impact on results.	
	Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced dispropor- tionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an in- fluence on the meanvalues of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathologicalchanges suggestive of tract im- pairment were noted inthose animals.	

Domain 7: Data Presentation and Analysis

Diethylhexyl Phthalate

		continu	ed from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	 Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i>; Adult Other (please specify below) (Organ Weight) 			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	630680			
Domain		Metric	Rating	Comments
	Metric 21:	Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group vari- ances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily ex- plained.

The authors reported effect on organ weight for several organs- liver, pancreas, testes and spleen. Additional Comments:

Overall Quality Determination

Medium

	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common						
Duration	marmosets:	narmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.					
Exposure Route	Terrestrial: F	Cood/Diet: Dietary	uays				
Media. Path:	Terrestriar, T	ood/Diet, Dietary					
Taxa. Species. Age:	Vertebrate: N	Aammalian: Common marmosets (Callithrix	: iacchus): Adult				
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	630680						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	9		_				
	Metric 1:	Test Substance Identity	Low	The chemical is identified as di(2-ethylhexyl)phthalate (DEHP), but no other identifying information is provided.			
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.			
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.			
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Char	Matria 7:	Experimental System/Test Media	High	DEIID was dissolved in some oil and administered analy, with a softester ones doily in			
	Metric 7.	Preparation	nıgıı	the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.			
	Metric 8:	Consistency of Exposure	High	The exposure solution volume or the number of molecules of the test substance per			
	Matria 0.	Administration Manuarement of Test Substance	Low	container were the same across replicates and groups.			
	Metric 9:	Concentration	Low	tions were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).			
	Metric 11:	Number of Exposure Groups/	High	Dosages of 2500, 500, and 100 mg/kg were chosen based on the results of range-finding tests conducted before the initiation of the definitive test			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure, so the solubility was not relevant as it was administered via gavage.			
Domain 4: Test Organism							
Domain 4. 1050 Organii5in	Metric 13:	Test Organism Characteristics	Medium	Test organisms were sourced from "CLEA JAPAN" and were separated by sex. Age of the test organisms was not specified, which could have affected the outcome of the test.			
	Metric 14:	Acclimatization and Pretreatment	High	Marmosets were acclimated for 3 months in the same conditions as the test.			
	Metric 15:	Conditions Number of Organisms and	Low	20 males and 20 females were selected and divided intofive groups equal in size. The			
		Replicates per Group		use of representation was unclearly described.			

Diethylhexyl Phthalate

continued from previous page					
Study Citation:	Kurata, Y., I marmosets: 1	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia, Toxicological Sciences 42(1):49-56.			
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: >	> 21 days		
Exposure Route,	Terrestrial; F	lood/Diet; Dietary			
Media, Path:					
Taxa, Species, Age:	Vertebrate; N	Iammalian; Common marmosets (Calli	thrix jacchus); Adult		
Health Outcome:	Developmen	t/Growth			
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	630680				
Domain		Metric	Rating	Comments	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for mar- mosets was maintained at a room temperature of $26 \pm 2^{\circ}$ C, a relative humidity of $50 \pm 10\%$, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh	

				cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and ad- ditives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given assupplemental food. Tap water passed through a 5-/xm filter andirradiated by ultraviolet was freely available to the animalsfrom water bottles.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. There are no limitations that would result in a substantial impact on results.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced dispropor- tionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an in- fluence on the meanvalues of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathologicalchanges suggestive of tract im- pairment were noted inthose animals.

Domain 7: Data Presentation and Analysis

Diethylhexyl Phthalate

Study Citation:				
Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Kurata, Y., I marmosets: 1 Overall Dura Terrestrial; F Vertebrate; N Developmen Di-ethylhexy 630680	Kidachi, F., Yokoyama, M., Toyota, N., Tsu Lack of hepatic peroxisome proliferation, test tion: > 21 days; Exposure Duration: > 21 d 'ood/Diet; Dietary Mammalian; <i>Common marmosets (Callithrix J</i> t/Growth '1 phthalate (DEHP)	uchitani, M., K ticular atrophy, ays <i>jacchus)</i> ; Adult	atoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.
Domain		Metric	Rating	Comments
	Metric 21:	Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group vari- ances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.

The authors reported the effect on growth by reporting the weight differences between the control and treatment groups after 13 weeks. As the age of the Additional Comments: test organisms was not provided, it was unclear whether this was an effect on growth or weight loss.

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus domesticus; Adult			
Health Outcome:	Behavioral	, , ,			
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	683058				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 2: Exposure Ch	aractorization				
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	High	The experimental system and the methods for preparation of the test media were de	
	Wettre 7.	Preparation	mgn	scribed in adequate detail.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
		Administration	U		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the feed for 4 weeks.	
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was tested.	
		Spacing of Exposure Levels			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.	
	16 . 17	Conditions			
	Metric 15:	Number of Organisms and	Medium	15 birds were used in each treatment group.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.	
		Contir	nued on next pa	ge	

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Diethylhexyl Phthalate

... continued from previous page Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156. Study Citation: **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Exposure Route, Media, Path: Vertebrate; Avian; Gallus domesticus; Adult Taxa, Species, Age: **Health Outcome:** Behavioral Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 683058 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology Medium The outcome assessment methodology reported the intended outcome of interest (food consumption), but the results were summarized for the treatment groups and not by individual animal. Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Medium Metric 19: The authors reported that the treatments affected food consumption compared to the Design and Procedures control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall. Metric 20: Outcomes Unrelated to Exposure Medium The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Statistical analysis was performed but not described adequately. Low Metric 22: Reporting of Data Low Data for exposure-related findings were presented for each treatment and control group, but measures of variability were not reported. Metric 23: Explanation of Unexpected Outcomes Low A measure of variability is not reported in Figure 1. Additional Comments: This form is for the data provided for feed consumption behavior. This is a behavioral endpoint, but it also a confounding variable in avian dietary toxicity studies. **Overall Quality Determination** Medium

Study Citation: Duration: Exposure Route, Media. Path:	Wood, D. L., Overall Dura Terrestrial; F	Bitman, J. (1980). The effect of feeding di- tion: > 21 days; Exposure Duration: > 21 d 'ood/Diet; Dietary	-(2-ethylhexyl) p days	hthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Reproductive Di-ethylhexy 683058	avian; <i>Gallus domesticus</i> ; Adult e/Teratogenic vl phthalate (DEHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the diet for 4 weeks.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4. Test Organisi	m			
Domain in 1600 organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The authors report that 15 chickens were used per treatment group.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

Diethylhexyl Phthalate

continued from previous page						
Study Citation: Duration: Exposure Route,	Wood, D. L. Overall Dura Terrestrial; F	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus domesticus; Adult				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	683058	683058				
Domain		Metric	Rating	Comments		
Domain 6: Confounding	/ Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior).		
Domain 7: Data Presenta	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group as % egg production, but no measure of variability was presented.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report a measure of variability for egg production.		
Additional Comments:	This evaluat	ion is for the egg production endpoint.				

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult Development/Growth			
Taxa, Species, Age:				
Health Outcome:				
Chemical:	Di-ethylhexyl phthalate (DEHP)			
HERO ID:	683058			
Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
Domain 21 Test Dosign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Denneir 2: Ennerme Okanasteriaetian				
Domain 5: Exposure Ch	Matria 7:	Experimental System/Test Media	Uich	The experimental system and the methods for properties of the test mode were de
	Metric 7:	Preparation	nign	scribed in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the diet for 4 weeks.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Authors reported that there were 15 animals used per treatment.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17: Metric 18:	Outcome Assessment Methodology	High High	The outcome assessment methodology reported the intended outcome of interest.
	wieute 10.	Assessment	Ingli	Outcomes were assessed consistently across study groups.
Diethylhexyl Phthalate

		contin	nued from p	revious page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Wood, D. L., Overall Dura Terrestrial; F Vertebrate; A Development Di-ethylhexy	Bitman, J. (1980). The effect of feeding d tion: > 21 days; Exposure Duration: > 21 ood/Diet; Dietary vian; <i>Gallus domesticus</i> ; Adult t/Growth 1 phthalate (DEHP)	i-(2-ethylhex days	yl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.
Domain	085058	Metric	Rating	Comments
Domain 6: Confounding	/ Variable Cor	ntrol	Truting	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior).
Domain 7: Data Presenta	ation and Analy	ysis		
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There do not appear to be any unexpected outcomes, and variability for body weight was reported. No measure of variability was reported for liver weight.
Additional Comments:	This evaluati	on form is for the growth endpoints of bod	y and liver w	reight.

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Wood, D. L. Overall Dura Terrestrial; F Vertebrate; A Mechanistic.	, Bitman, J. (1980). The effect of feeding di-(ation: > 21 days; Exposure Duration: > 21 d Food/Diet; Dietary Avian; <i>Gallus domesticus</i> ; Adult -Biomarkers (exposure and effect)	(2-ethylhexyl) j ays	phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.
Chemical:	Di-ethylhexy	yl phthalate (DEHP)		
HERO ID:	683058			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2. Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were de- scribed in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the diet for 4 weeks.
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was tested.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organis	Matria 12:	Test Organism Characteristics	Uich	The test experience were adapted to described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms
	Metric 14.	Conditions	Ingn	retreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and	Low	The number of test organisms and the number of replicates were lower than the typical
		Replicates per Group		number used in studies of the same or similar type. Only 4 hens were used to examine lipid and cholesterol levels.
Domain 5: Outcome As	sessment			
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment	0	
		Contin	ued on next pa	age

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Diethylhexyl Phthalate

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Wood, D. L. Overall Dura Terrestrial; F Vertebrate; A Mechanistic Di-ethylhexy 683058	, Bitman, J. (1980). The effect of feeding di- ation: > 21 days; Exposure Duration: > 21 o Food/Diet; Dietary Avian; <i>Gallus domesticus</i> ; Adult -Biomarkers (exposure and effect) yl phthalate (DEHP)	(2-ethylhexyl) p days	ohthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.	
Domain	Metric Rating Comments				
Domain 6: Confoundin	g / Variable Co Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Medium Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall. The authors reported that the treatments affected food consumption compared to the control (aversion behavior).	
Domain 7: Data Presen	tation and Anal Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	Low High Low	Statistical analysis was performed but not described adequately. Data for exposure-related findings were presented for each treatment and control group. No measure of variability was presented for the liver and plasma endpoints (authors state n = 4 per treatment for these endpoints).	

Additional Comments: This evaluation form is for the plasma and liver chemistry endpoints (cholesterol and lipid %).

Overall Quality Determination

Medium

Study Citation:	Abdul-Ghani	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A di-butyl Phthalate (DBP) in a chick model	., Abdeen, Z.	. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate					
Duration:	Overall Dura	ition: Not-reported; Exposure Duration: N	ot-reported	biogy and relations y 54(1).50-02.					
Exposure Route, Modia Bath	Terrestrial; N	VA (e.g., injection); Injection							
Taxa, Species, Age:	Vertebrate: A	vian: Gallus gallus domesticus: Cobb broi	iler strain: Er	nbryo					
Health Outcome:	Mortality	,	,						
Chemical:	Di-ethylhexy	l phthalate (DEHP)							
HERO ID:	1249807								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce		TT' 1						
	Metric 1: Matria 2:	Test Substance Identity	High	The chemical was identified by name.					
	Metric 2:	Test Substance Purity	Low High	The test substance identity was not analytically verified by the performing laboratory.					
	Metric 5.	Test Substance I unity	Ingn	Chemical purity was reported as 99.170.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.					
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.					
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.					
Domain 3: Exposure Ch	aracterization								
1	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.					
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.					
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.					
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.					
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.					
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.					
Domain 4: Test Organis	m								
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.					
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.					
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient					
		Replicates per Group		to characterize toxicological effects.					
Domain 5: Outcome Ass	recoment								
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.					
		Conti	inued on nex	xt page					
			-						

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Diethylhexyl Phthalate

		contin	ued from p	previous page	
Study Citation:	Abdul-Ghan	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A.	, Abdeen, Z	. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate	
Duration: Exposure Route,	Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; N/A (e.g., injection); Injection				
Media, Path: Taxa, Species, Age:	Vertebrate: Avian: Gallus gallus domesticus: Cobb broiler strain: Embryo				
Health Outcome:	Mortality	, , ,	,	,	
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	1249807				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.	
		Assessment			
Domain 6: Confounding	g / Variable Cor	ntrol			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.	
		Design and Procedures			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no outcomes unrelated to exposures among study groups.	
Domain 7: Data Present	ation and Anal	ysis	II: -L		
	Metric 21: Matria 22:	Statistical Methods Reporting of Data	High	Statistical methods were adequately described.	
	Metric 22: Metric 23:	Explanation of Unexpected Outcomes	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 25.	Explanation of Onexpected Outcomes	Ingn	There were no unexpected outcomes.	
Additional Comments:	This mortali	ty form was added for hatch.			
Overall Quali	ty Detern	nination	High		

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Study Citation:	Abdul-Ghan	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A	, Abdeen, Z.	(2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate
Duration: Exposure Route,	(DEHP) and Overall Dura Terrestrial; N	di-butyl Phthalate (DBP) in a chick model ation: Not-reported; Exposure Duration: N J/A (e.g., injection); Injection	l. Neurotoxic ot-reported	ology and Teratology 34(1):56-62.
Media, Path: Taya Species Age:	Vertebrate: A	avian: Gallus gallus domesticus: Cobb bro	iler strain: Fn	nhrvo
Health Outcome:	Developmen	t/Growth	nei suani, En	libryo
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	1249807			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce		TT' 1	
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2: Metric 3:	Test Substance Purity	Low High	The chemical purity was reported as 99.7%
	mente J.	Test Substance Fully	mgn	The element purity was reported as 77.170.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 2. Evenance Ch	anastanization			
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare
	Wietife 7.	Preparation	LOW	test concentrations.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and the spacing of exposure levels were adequate for a does represe
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via egg injection
		g at of Doroth Bolaonity Ennit	1.1/1	
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
				<u> </u>
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment		

Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Abdul-Ghani, S., Yanai, J., Abdul-Ghani, R., Pinkas, A., Abdeen, Z. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate (DEHP) and di-butyl Phthalate (DBP) in a chick model. Neurotoxicology and Teratology 34(1):56-62. **Duration:** Overall Duration: Not-reported; Exposure Duration: Not-reported **Exposure Route**, Terrestrial; N/A (e.g., injection); Injection Media, Path: Taxa, Species, Age: Vertebrate; Avian; Gallus gallus domesticus; Cobb broiler strain; Embryo **Health Outcome:** Development/Growth Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 1249807 Domain Metric Rating Comments Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. Design and Procedures High Metric 20: Outcomes Unrelated to Exposure There were no outcomes unrelated to exposures among study groups. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were adequately described. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Metric 23: High **Explanation of Unexpected Outcomes** There were no unexpected outcomes. Additional Comments: This form was for hatch defects.

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path:	Abdul-Ghani (DEHP) and Overall Dura Terrestrial; N	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A di-butyl Phthalate (DBP) in a chick model tion: Not-reported; Exposure Duration: N I/A (e.g., injection); Injection	, Abdeen, Z. l. Neurotoxic ot-reported	. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate ology and Teratology 34(1):56-62.				
Taxa, Species, Age:	Vertebrate; A	vian; Gallus gallus domesticus; Cobb bro	iler strain; En	nbryo				
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)							
Chemical:	Di-ethylhexy	Di-ethylnexyl phthalate (DEHP)						
	1249607							
Domain 1: Test Substan	20	Metric	Rating	Comments				
Domain 1. Test Substand	Metric 1.	Test Substance Identity	High	The chemical was identified by name				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	The chemical substance purity was reported as 99.7%.				
		•	<u> </u>					
Domain 2: Test Design			TT: 1					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5: Matria 6:	Regative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 0.	Kandonnized Anocation	LOW	Researchers and not report now organisms were anocated to study groups.				
Domain 3: Exposure Ch	aracterization							
1	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare				
		Preparation		test concentrations.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one test concentration and a control were used.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.				
Domain 4: Test Organisi	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient				
		Replicates per Group						
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
	Continued on next page							

Diethylhexyl Phthalate

	continued from previous page				
Study Citation:	Abdul-Ghan	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A. di-butyl Phthalate (DBP) in a chick model	, Abdeen, Z	. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate	
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: No	t-reported		
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Injection	1		
Media, Path:					
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus gallus domesticus; Cobb broil	er strain; Ei	nbryo	
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Genotox	(including	DNA repair)	
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	1249807				
Domain		Metric	Rating	Comments	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.	
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	There were no outcomes unrelated to exposures among test groups.	
Domain 7: Data Present	tation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This evaluat	on form was for DNA damage.			
Overall Quali	ty Detern	nination	High		

Study Citation:	Abdul-Ghan	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A	., Abdeen, Z.	(2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate
Duration:	(DEHP) and Overall Dura	di-butyl Phthalate (DBP) in a chick mode ation: Not-reported: Exposure Duration: N	 Neurotoxic ot-reported 	ology and Teratology 34(1):56-62.
Exposure Route,	Terrestrial; N	J/A (e.g., injection); Injection	et reponted	
Media, Path:	,	(, g, j, , , , j, j, , , , , , , , , , ,		
Taxa, Species, Age:	Vertebrate; A	vian; Gallus gallus domesticus; Cobb bro	iler strain; En	nbryo
Health Outcome:	Behavioral			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	1249807			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.7%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
	, . , .			
Domain 3: Exposure Ch	aracterization		Ŧ	
	Metric /:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	N/A	Only one test concentration and a control were used.
		Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.
Domain 4: Test Organisi	m			
0	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms and the number of replicates were reported and sufficient
		Replicates per Group		to characterize toxicological effects.
Domain 5: Outcome Ass	sessment			
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of or- ganism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment	•1	
		Cont	inued on nex	tt page

Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Abdul-Ghan	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A.	, Abdeen, Z	. (2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate			
	(DEHP) and	di-butyl Phthalate (DBP) in a chick model.	Neurotoxic	cology and Teratology 34(1):56-62.			
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported						
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Injection					
Media, Path:							
Taxa, Species, Age:	Vertebrate; A	vian; Gallus gallus domesticus; Cobb broil	er strain; Ei	mbryo			
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	1249807						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no outcomes unrelated to exposures among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluati	on form is for activity and imprinting.					

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Herreros, M. A., Encinas, T., Torres-Rovin, L., Garcia-Fernandez, R. A., Plores, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine discrptifyed (cit_entyhtybulnata effects features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 30(3):1141-1149. Duration: Overall Duration: > 21 days. Exposure Route, Terrestrial: N/A (e.g., injection) Injection) Injection Hedia, Path: Taras, Species, Age: Vertebrate: Mammalian, Ovis arise; Manchega breed; Adult Health Outcome: Hepath:Outcome: Hepath: Outcome: Domain Metric Rating Domain Metric Rating Domain Metric Rating Domain 1: Test Substance Low The test substance vas identified by name only. Metric 2: Test Substance lentity Low The test substance vas identified by name only. Domain 2: Test Design Metric 4: Negative Controls High Metric 5: Negative Controls High Study aubors reported using six sheep as negative controls that received salia ingic-tions. Domain 3: Exposure Characterization Ketric 6: Negative Controls High Metric 6: Negative Controls High Study aubors reportend using s								
bratalon:: Overall Duration: > 21 days: Exposure Duration: > 21 days Exposure Route, Terrestrial: N/A (e.g., injection): fijection) Media, Path: Taxa, Species, Age: Domain Hepatic/Liver Chemical: Domain Metric 1: Test Substance function: Metric 1: Test Substance Survey Metric 2: Test Substance Survey Metric 2: Test Substance Survey Metric 2: Negative Controls: Metric 1: Test Substance Survey Metric 2: Negative Controls: Metric 1: Test Substance Survey Metric 2: Negative Controls: Metric 3: Test Substance Survey Metric 4: Negative Controls: Metric 5: Negative Controls: Metric 6: Negative Control Response: Metric 6: Negative Control Response: Metric 6: Negative Control Response: Metric 7: Test Substance Media: Metric 8: Consistency of Exposure Metric 10: Consistency of Exposure Metric 10: Consistency of Exposure Metric 11: Number of Exposure Const. Metric 12: Test of Substance Low Metric 12: Test of Substance Cource All Media: Metric 12: Test of Substance Cource All Media: Metric 12: Test of Consistency of Exposure All Media: Metric 12: Test of Substance Cource All Media: Metric 12: Test of Substance Cource All Media: Metric 12: Test of Consistency of Exposure All Media: Metric 12: Test of Resposure Duration and Prequency High Metric 13: Test Organism Characteristics	Study Citation:	Herreros, M	. A., Encinas, T., Torres-Rovira, L., Garcia-	Fernandez, R. A.	, Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine			
Duration: Overall Duration: > 21 days Exposure Route, Terrestrial: NA (e.g., injection); fnjection Media, Palit Terrestrial: NA (e.g., injection); fnjection Media, Palit Heatth Outcome: Palitica, Palita, Palita, Palitica, Palitica, Palita, Palitica, Palitica, Pali		disruptor di	(2-ethylhexyl)phthalate affects female repro	oductive features	by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology			
Duration: Overall Duration: 2 It days reports Exposure Route, Media, Path: Terrestrial: V/A (cg., injection): Injection): Vial days injection injection injection Taxa, Species, Age: Vertebrate; Mammalian; Ovis aries; Manchega breed; Adult Health Outcome: Dimain Dimain (injection): Dimain (injection): Injection (injection): Domain Metric Rating Comments Domain 1: Test Substance Junce Identity Low The test substance was identified by name only. Metric 1: Test Substance Purity Low The source of the DEHP was not reported. Domain 2: Test Design Metric 4: Negative Controls High Metric 6: Ragitive Controls High The regative control response was reported in Figure 3. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Media Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Preparation Metric 7: Reparimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 7: Reparementalon Metric 8: Con	Demotions	36(3):1141-	1149. - ti-ne > 21 daves Essa and Durations > 21	4				
Paper Route, Media, Path Infection (migration) Media, Path Vertebrate; Mammalian; Ovis aries; Manchega breed; Adult Health Outcome Hepath(action) Domain Metric Rating Comments Domain Metric Rating Comments Domain 1: Test Substance Metric Test Substance Vertice Hepath Domain 1: Test Substance Metric 2: Test Substance Source Low The test substance was identified by name only. Metric 2: Test Substance Source Low The source of the DEIP was not reported. Metric difference Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 6: Randomized Allocation Medium Stresponse was reported in Figure 3. Metric 7: Reperimental System/Test Media Low The preparation of the DEIP injections was not reported. Metric 6: Randomized Allocation Medium Exposure swere administration were not coported. Metric 7: Preparation Metric 7: Exposure formation of the DEIP injections was not reported. </th <th>Duration:</th> <th>Overall Dura</th> <th>ation: > 21 days; Exposure Duration: > 21</th> <th>days</th> <th></th>	Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Methy Path Vertebrate: Mammalian: Ovis aries: Manchega breed; Adult Health Outcome Hepatio/Liver Domain Metric Rating Comments Domain Metric Rating Comments Domain Metric Resource of the Metric Comments Domain Metric 1: Test Substance Comments Metric 2: Test Substance Identity Low The test substance was identified by name only. Metric 3: Test Substance Purity Low The resource of the DEHP was not reported, nor was it analytically verified. Domain 2: Test Design Metric 5: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Metric 5: Reparation Metric 1: Exposure Paration Metric 6: Metric 6: Randomized Allocation Medium Stopsare administered via intramuscular injection 3x a week for 2 months. Other defains regarding the cynoare administration was neasured. Metric 6: Resposure Duration and Frequency High	Exposure Route,	Terrestrial; T	N/A (e.g., injection); injection					
Taxa, species, Age: verteorate: Manimulan: Ors artes: Manchega breed; Aduit Health Outcome: Hepatholicit.Vier Chemical: Di-thylhexyl phthalate (DEHP) HERO ID: 2519005 Domain Metric 1: Test Substance The start substance was identified by name only. Metric 2: Test Substance Identify Low Metric 3: Test Substance Parity Low Domain 2: Test Design Metric 6: Radive Controls Metric 5: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization The spectrace and ministration was not reported. Preparation of the DEHP injections was not reported. Metric 9: Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 9: Metric 1: Storgarding the exposure administration was reported. Administration Admin	Media, Path:	X7 (1 ()		. 1 1				
Heantin Outcome: Prepatitic/Liver prepatition: Directlyftexcy philabilities (DEHP) Domain Directlyftexcy philabilities (DEHP) HERO ID: 2519005 Domain 1: Test Substance Lefter (1: Test Substance Identify Low The test substance was identified by name only. Metric 2: Test Substance Purity Low The Issues and the DEHP was not reported, nor was it analytically verified. Metric 3: Test Substance Purity Low The DEHP purity was not reported, nor was it analytically verified. Metric 5: Negative Controls High Study authors reported using six sheep as negative controls that received saline injec- tions. Metric 6: Negative Controls High The negative control response was reported in Figure 3. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Preparation Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Preparation Metric 8: Consistency of Exposure Medium Exposures were administered via intramuscular injection 3: a week for 2 months. Other daministration Austrement of Test Substance Low It was not reported if the exposure concentration was measured. Concentration Metric 10: Exposure Groups/ Metric 11: Number of Exposure Groups/ Metric 12: Testing at or Below Solubility Limit N/A The exposure was via injection. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Sheep were reported to be from the experimental farm of the INA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in hadarid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study. Metric 14: Acclimatization and Pretreatment Low It was not reported to be from the experimental farm of the INA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the stud, Number of Organisms and Low Th	Taxa, Species, Age:	vertebrate; r	Mammalian; Ovis aries; Manchega breed; A	Adult				
Chemical: Dreaminest (DEP) Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low The test substance only include the point of the DEIP was not reported, nor was it analytically verified. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections and there is a substance was identified by name only. Domain 2: Test Design Metric 6: Randomized Allocation High Study authors reported using six sheep as negative controls that received saline injections and there is a substance was identified by name only. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 9: Experimental System/Test Media Low The preparation of the DEIP injections was not reported. Metric 9: Measurement of Test Substance Low It was not reported to intranuscular injection 3: a week for 2 months. Other details regaring the exposure durinisation was measured. Concentration frequency Metric 10: Exposure Duration and Frequency High The exposure duration to skeer exposure period. the follicular phase was observed. This appeared to be an afequate duration to observe	Chamical	D: athall	i-ethylhexyl phthalate (DEHP)					
Intervolution Z519003 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low The test substance was identified by name only. Metric 2: Test Substance Source Low The source of the DEHP was not reported, nor was it analytically verified. Domain 2: Test Substance Source High Study authors reported using six sheep as negative controls that received saline injections. Metric 3: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 9: Consistency of Exposure Medium Exposures were administration were not reported. Preparation Metric 11: Number of Exposure Groups/ High The exposure duration was neasured. The exposure duration was not ported. Metric 12: Testing at or Below Studyauthors reported if the exposure preorid,	UEDO ID.	Di-ethylnex	<i>n</i> -einyinexyi phinaiale (DEHP)					
Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Source Low The test substance was identified by name only. Metric 3: Test Substance Source Low The bource of the DEHP was not reported, nor was it analytically verified. Metric 3: Test Substance Purity Low The DEHP was not reported. Domain 2: Test Design Metric 4: Negative Controls High Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization The regative control response was not reported. Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 9: Consistency of Exposure Medium Exposure administration were not reported. Metric 9: Measurement of Test Substance Low It was not reported in the preparation of the DEHP injections was not reported. Metric 11: Number of Exposure Medium <td< th=""><th>HERO ID:</th><th>2519005</th><th></th><th></th><th></th></td<>	HERO ID:	2519005						
Domain 1: Test Substance Metric 1: Test Substance Identity Low The test substance was identified by name only. Metric 2: Test Substance Source Low The source of the DEHP was not reported, nor was it analytically verified. Domain 2: Test Design Metric 3: Test Substance Purity Low The DEHP purity was not reported. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 8: Consistence y of Exposure Medium Exposure swere administration were not reported. Metric 9: Measurement of Test Substance Low It was not reported to the aposure of months. Other details regarding the exposure duration was neasured. Metric 10: Concentration Exposure Duration and Frequency High The exposure duration was net peorted to be 2 months with intramuscular injections 3x a week for 2 months. Other datails regarding the exposure duration to observe a response. Metric	Domain		Metric	Rating	Comments			
Metric 1: Test Substance Identity Low The test substance was identified by name only. Metric 2: Test Substance Source Low The bettP was not reported, nor was it analytically verified. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High Study authors reported using six sheep as negative controls that received saline injections. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation The preparation of the DEHP injections was not reported. Metric 9: Metric 0: Consistency of Exposure Administration Medium Exposures were administration were not reported. Metric 10: Exposure Duration and Frequency High The exposure duration was reported to be 2 months. Other details regarding the exposure concentration was measured. Metric 11: Number of Exposure Groups/ Spacing of Exposure Groups/ Metric 12: N/A The exposure duration was reported to be 2 months with intranuscular injections 3x a week. After the exposure action. Domain 4: Test Organism Metric 13: Test Organism Characteristics Hi	Domain 1: Test Substand	ce		Ŧ				
Metric 2: Test Substance Source Low The source of the DEHP purity was not reported, nor was it analytically verified. Metric 3: Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 6: Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Preparation Metric 6: Randomized Allocation Medium Exposures were administered via intranuscular injection 3x a week for 2 months. Other Administration Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 8: Consistency of Exposure Medium Exposures were administered via intranuscular injection 3x a week for 2 months. Other datalis regarding the exposure administration was neasured. Metric 10: Exposure Duration and Frequency High The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response. Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels N/A The exposure was via injection. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported dudit, fenale s		Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
Metric 3: Fest Substance Purity Low The DEHP purity was not reported. Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Medium Domain 3: Exposure Characterization Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Preparation Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 8: Consistency of Exposure Medium Exposures were administered via intramuscular injection 3x a week for 2 months. Other details regarding the exposure administration was neot neported. Metric 9: Measurement of Test Substance Low It was not reported if the exposure concentration was measured. Metric 10: Exposure Duration and Prequency High The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe are seponse. Metric 11: Number of Exposure G		Metric 2:	Test Substance Source	Low	The source of the DEHP was not reported, nor was it analytically verified.			
Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Low The preparation of the DEHP injections was not reported. Metric 8: Consistency of Exposure Administration Medium Exposures were administration were not reported. Metric 9: Metasurement of Test Substance Low It was not reported if the exposure concentration was measured. Metric 10: Exposure Duration and Frequency High The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure proid, the folicular phase was observed. This appeared to be an adequate duration to observe a response. Metric 11: Number of Exposure Groups/ Spacing of Exposure Curvels N/A The exposure was via injection. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were		Metric 3:	Test Substance Purity	Low	The DEHP purity was not reported.			
Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using six sheep as negative controls that received saline injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Low The preparation of the DEHP injections was not reported. Metric 8: Consistency of Exposure Administration Medium Exposures were administration were not reported. Metric 9: Measurement of Test Substance Low It was not reported if the exposure concentration was measured. Metric 10: Exposure Groups/ Spacing of Exposure Groups/ Metric 11: N/Metro of Exposure Groups/ Spacing of Exposure Levels N/A The exposure was via injection. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Number of Capanism Characteristics High N/A Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study. Metric 14: Acclimatization and Pretreatment Conditions Low There were 6 sheep in the control and 8 sheep in the tre	Domain 2: Test Design							
Metric 4. Regarive Controls Fright Study autions reported using six sheep as negative controls that received same injections. Metric 5: Negative Control Response High The negative control response was reported in Figure 3. Metric 6: Randomized Allocation Medium Sheep were randomly allocated into control or treatment groups. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Low The preparation of the DEHP injections was not reported. Metric 8: Consistency of Exposure Medium Exposures were administration was neasured. Metric 9: Measurement of Test Substance Low It was not reported to be 2 months with intramuscular injections 3x a week for 2 months. Other details regarding the exposure concentration was measured. Metric 10: Exposure Duration and Frequency High The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure concentration to baserve a response. Metric 11: Number of Exposure Groups/ N/A The goal of the study was not to have a dose response, but to observe one treatment level and compare it to a control. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega bree	Domain 2. Test Design	Matria 4	Nagativa Controls	High	Study outhous concerted using six shape as possible controls that esseived called inice			
Metric 5: Metric 6:Negative Control Response Randomized AllocationHigh MediumThe negative control response was reported in Figure 3. Sheep were randomly allocated into control or treatment groups.Domain 3:Exposure Characterization Metric 7:Experimental System/Test Media PreparationLowThe preparation of the DEHP injections was not reported. Preparation of the DEHP injections was not reported. PreparationMetric 8:Consistency of Exposure Administration Metric 9:MediumExposures were administered via intranuscular injection 3x a week for 2 months. Other details regarding the exposure administration were not reported. It was not reported if the exposure concentration was measured. Concentration Metric 10:Exposure Orest SubstanceLowIt was not reported if the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response.Metric 11:Number of Exposure Groups/ Spacing of Exposure Groups/ Spacing of Exposure Levels Metric 12:N/AThe exposure was via injection.Domain 4:Test OrganismMetric 13:Test Organism CharacteristicsHigh MighMetric 14:Acclimatization and Pretreatment Conditions Metric 15:LowIt was not reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported to be from the sheep of the Manchega breed were used in the study.		Meure 4.	Negative Controls	rigii	tions.			
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Metric 14: Acclimatization and Pretreatment Low It was not reported if the sheep were acclimated prior to the start of the test. Conditions Conditions Low There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size.		Metric 13:	Test Organism Characteristics	High	Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study.			
Metric 15: Number of Organisms and Low There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size.		Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the sheep were acclimated prior to the start of the test.			
Replicates per Group sample size.		Metric 15:	Conditions Number of Organisms and	Low	There were 6 sheep in the control and 8 sheep in the treatment group. This is a small			
			Replicates per Group		sample size.			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Injection					
Media, Path:							
Taxa, Species, Age:	Vertebrate; N	Mammalian; Ovis aries; Manchega breed; Ad	dult				
Health Outcome:	Hepatic/Live	er					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2519005						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in liver tissue.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Liver tissue was fixed and examined via light microscopy.			
Domain 6: Confounding	/ Variable Co	ntrol					
C	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.			
	Metric 22:	Reporting of Data	High	Exposure and control data were presented in Figure 3 and were adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes.			

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Toxa, Species, Age:	Herreros, M disruptor di(36(3):1141- Overall Dura Terrestrial; N	A., Encinas, T., Torres-Rovira, L., Garcia- (2-ethylhexyl)phthalate affects female repro- 1149. ation: > 21 days; Exposure Duration: > 21 N/A (e.g., injection); Injection	Fernandez, R. A., oductive features days	, Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology				
Health Outcome:	Mechanistic	Mechanistic-Endocrine toxicity						
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2519005	-						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DEHP was not reported, nor was it analytically verified.				
	Metric 3:	Test Substance Purity	Low	The DEHP purity was not reported.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using six sheep as negative controls that received saline injec- tions.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 1 and 2 and was adequate for the outcomes of interest.				
	Metric 6:	Randomized Allocation	Medium	Sheep were randomly allocated into control or treatment groups.				
Domain 2. Expansion Ch	anastanization							
Domain 5: Exposure Ch	Metric 7.	Experimental System/Test Media	Low	The preparation of the DEHP injections was not reported				
	Weute 7.	Preparation	Low	The preparation of the DETH injections was not reported.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered via intramuscular injection 3x a week for 2 months. Other details regarding the exposure administration were not reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentration was measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response.				
	Metric 11:	Number of Exposure Groups/	N/A	The goal of the study was not to have a dose response, but to observe one treatment level and compare it to a control				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via injection.				
		· · · · · ·						
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the sheep were acclimated prior to the start of the test.				
	Metric 15:	Conditions Number of Organisms and Parliantee per Group	Low	There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size				
		Replicates per Oroup		sumple size.				

Domain 5: Outcome Assessment

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Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Herreros, M disruptor di(36(3):1141-	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route.	Terrestrial: N/A (e.g., injection): Injection						
Media, Path:	,						
Tava Species Age	Vertebrate: N	Vertebrate: Mammalian: Ouis arias: Manchega breed: Adult					
Hoolth Outcome:	Machanistic	Endocrine toxicity	uun				
Chamicali	Di athulhavi	ul phthalata (DEHD)					
UEBO ID.	2510005	yi philialate (DEFF)					
HERO ID:	2519005						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–changes in estradiol and progesterone levels.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Enzyme immunoassay kits were used to determine hormone levels.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	lysis					
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.			
	Metric 22:	Reporting of Data	High	Exposure and control data were presented in Figures 1 and 2 and were adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability was reported in the figures.			
Additional Comments:	This portion the outcome	of the evaluation was on the effect of DEHI of interest.	P on plasma estr	adiol and progesterone levels. The endocrine mechanistic outcome was chosen as			

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocr disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacolog 36(3):1141-1149. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; N/A (e.g., injection); Injection						
Media, Path:	Vartabrata, N	Mammalian, Quis guise Manahaga huadi A	dult				
Health Outcome	Reproductiv	vianimanan, Ovis aries; Manchega breed; A	aun				
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2519005						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DEHP was not reported, nor was it analytically verified.			
	Metric 3:	Test Substance Purity	Low	The DEHP purity was not reported.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using six sheep as negative controls that received saline injec- tions.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 1 and 2.			
	Metric 6:	Randomized Allocation	Medium	Sheep were randomly allocated into control or treatment groups.			
Damain 2: European Ch							
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DEHP injections was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered via intramuscular injection 3x a week for 2 months. Other details regarding the exposure administration were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentration was measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The goal of the study was not to have a dose response, but to observe one treatment level and compare it to a control.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via injection.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the sheep were acclimated prior to the start of the test.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size.			

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Diethylhexyl Phthalate

		contin	ued from previ	ous page				
Study Citation:	Herreros, M disruptor di(36(3):1141-2	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.						
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	lays					
Exposure Route,	Terrestrial; N	Terrestrial; N/A (e.g., injection); Injection						
Media, Path:								
Taxa, Species, Age:	Vertebrate: Mammalian: Ovis aries: Manchega breed: Adult							
Health Outcome:	Reproductiv	Reproductive/Teratogenic						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID.	2519005	(DEFIL)						
	2517005							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in the ovaries due to DEHP exposure.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Ovarian structures were examined via ultrasound, and after that, they were fixed and examined via light microscopy.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Presentation and Analysis								
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.				
	Metric 22:	Reporting of Data	High	Exposure and control data were presented in Figures 1 and 2 and were adequate for the outcomes of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability was reported in the figures.				

This portion of the evaluation was on the effect of DEHP on ovarian structure in Manchega sheep. Reproduction was selected as the outcome of interest. Additional Comments:

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Lake, B. G.,	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate				
Duration: Exposure Route, Media. Path:	in the ferret. Overall Dura Terrestrial; F	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Hepatic/Live Di-ethylhexy 746754	Mammalian; <i>Putorius putorius</i> ; Adult er yl phthalate (DEHP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].		
	Metric 2:	Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British PetroleumChemicals International Ltd., Epsom, Surrey."		
	Metric 3:	Test Substance Purity	High	DEHP was reported to have >99% purity, which was verified by GLC.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A concurrent negative control was used.		
	Metric 5:	Negative Control Response	High	Biological responses in the negative control were normal.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.		
	Metric 8:	Consistency of Exposure Administration	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.		
	Metric 9:	Measurement of Test Substance Concentration	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.		
	Metric 10:	Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This was a single dose study (1% DEHP w/w diet).		
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure.		
Domain 4: Test Organis	m					
-	Metric 13:	Test Organism Characteristics	High	"Male albino ferrets (Putorius putorius) were obtained from theWellcome Veterinary Re- search Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually ma- ture."		
		(Continued on next page			

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Diethylhexyl Phthalate

		con	tinued from previou	s page			
Study Citation:	Lake, B. G.,	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate					
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21 day	vs				
Exposure Route.	Terrestrial: F	Food/Diet: Dietary	, 5				
Media, Path:	, -						
Taxa, Species, Age:	Vertebrate; N	Vertebrate: Mammalian: <i>Putorius putorius</i> : Adult					
Health Outcome:	Hepatic/Live	er					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	746754						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods were detailed and were appropriate for the outcomes of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, & were assessed consistently (after organisms were killed at conclusion of study).			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	tation and Anal	veic					
	Metric 21.	Statistical Methods	High	Student's T test was used			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This study d	id not adequately measure the animals' intake	of DEHP, rendering it	unacceptable.			

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 746754 Table: 2 of 4

Study Citation: Duration: Exposure Route, Media Path:	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome:	Vertebrate; I Mechanistic Respiratory	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult Mechanistic-Cytotoxicity-Cardiovascular-Endocrine toxicity-Gastrointestinal-Kidney/renal-Liver toxicology-Neurotoxicology-Reproductive/Teratogenic- Respiratory					
Chemical: HERO ID:	Di-ethylhex 746754	yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].			
	Metric 2:	Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British PetroleumChemicals International Ltd., Epsom, Surrey."			
	Metric 3:	Test Substance Purity	High	The DEHP was reported to have >99% purity, which was verified by GLC.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A concurrent negative control used.			
	Metric 5:	Negative Control Response	High	Biological responses in the negative control were normal.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
Domain of Exposure of	Metric 7:	Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.			
	Metric 8:	Consistency of Exposure Administration	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.			
	Metric 9:	Measurement of Test Substance	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.			
	Metric 10:	Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.			
	Metric 11:	Number of Exposure Groups/	N/A	This was a single dose study (1% DEHP w/w diet).			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This was a dietary exposure.			
Domain 4. Test Organis	m						
Somani 4. Test Organis	Metric 13:	Test Organism Characteristics	High	"Male albino ferrets (Putorius putorius) were obtained from the Wellcome Veterinary Re- search Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually ma- ture."			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.			
		С	ontinued on next page .				

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

		con	tinued from previou	s page			
Study Citation:	Lake, B. G., in the ferret.	Brantom, P. G., Gangolli, S. D., Butterworth, K Toxicology 6(3):341-356.	K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate			
Duration:	Overall Dura	Dverall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; N	Mammalian; Putorius putorius; Adult					
Health Outcome:	Mechanistic	-Cytotoxicity-Cardiovascular-Endocrine toxicit	ty-Gastrointestinal-K	idney/renal-Liver toxicology-Neurotoxicology-Reproductive/Teratogenic-			
Chemical: HERO ID:	Respiratory Di-ethylhexy 746754	yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods were detailed and were appropriate for the outcomes of interest.			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, & were assessed consistently			
		Assessment		(after organisms were killed at conclusion of study).			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	The cell morphology assessment didn't need statistical analysis.			
	Metric 22:	Reporting of Data	Medium	Some of the cell morphology results were shown in figures. More was described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This study d	id not adequately measure the animals' intake	of DEHP, rendering i	unacceptable.			

Overall Quality Determination

Uninformative

Study Citation:	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.				
Duration: Exposure Route, Media. Path:	Terrestrial; I	Food/Diet; Dietary	days		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Reproductiv Di-ethylhexy 746754	Mammalian; <i>Putorius putorius</i> ; Adult e/Teratogenic yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].	
	Metric 2:	Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British PetroleumChemicals International Ltd., Epsom, Surrey."	
	Metric 3:	Test Substance Purity	High	The DEHP was reported to have >99% purity, which was verified by GLC.	
Domain 2: Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	A concurrent negative control was used.	
	Metric 5:	Negative Control Response	High	Biological responses in the negative control were normal.	
_	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
Domani 5. Exposure Cr	Metric 7:	Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.	
	Metric 8:	Consistency of Exposure Administration	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.	
	Metric 9:	Measurement of Test Substance	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.	
	Metric 10:	Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This was a single dose study (1% DEHP w/w diet).	
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	"Male albino ferrets (Putorius putorius) were obtained from the Wellcome Veterinary Re- search Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually ma- ture."	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.	
		(Continued on next page .		

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Diethylhexyl Phthalate

		cont	tinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Usalth Outcomes	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	746754					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on the results.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods were detailed and were appropriate for the outcomes of interest.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported & were assessed consistently (after organisms were killed at conclusion of study).		
Domain 6: Confounding	/ Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.		
Domain 7: Data Present	ation and Analy	veje				
Domain 7. Data Meselli	Metric 21:	Statistical Methods	High	Student's T test was used.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This study di	id not adequately measure the animals' intake of	of DEHP, rendering it	unacceptable.		

Overall Quality Determination

Uninformative

Study Citation:	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.				
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; I	ation: > 21 days; Exposure Duration: > 21 Food/Diet; Dietary	days		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Developmen Di-ethylhexy 746754	Mammalian; <i>Putorius putorius</i> ; Adult tt/Growth yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].	
	Metric 2:	Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British PetroleumChemicals International Ltd., Epsom, Surrey."	
	Metric 3:	Test Substance Purity	High	The DEHP purity was >99%, and it was verified by GLC.	
Domain 2: Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	A concurrent negative control was used	
	Metric 5:	Negative Control Response	High	Biological responses of the negative control were normal.	
_	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 2: Exposure Ch	aractorization				
Domani 5. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.	
	Metric 8:	Consistency of Exposure	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.	
	Metric 9:	Measurement of Test Substance	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.	
	Metric 10:	Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This was a single dose study (1% DEHP w/w diet).	
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	"Male albino ferrets (Putorius putorius) were obtained from the Wellcome Veterinary Re- search Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually ma- ture."	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre- treatment conditions were the same for control and exposed groups.	
		(Continued on next page .		

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Diethylhexyl Phthalate

		con	tinued from previous	page			
Study Citation:	Lake, B. G.,	Brantom, P. G., Gangolli, S. D., Butterworth, K Toxicology 6(3):341-356	K. R., Grasso, P. (1976)	. Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate			
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21 day	VS				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary					
Media, Path:		· · ·					
Taxa, Species, Age:	Vertebrate; N	Vertebrate; Mammalian; Putorius putorius; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	746754						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Frequency of weighing was not given & can only be estimated from Fig. 1.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were confusing, limited, or not reported.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. In particular, the loss of body weight from DEHP-treated food given ad libitum begs the question of palatability, and whether animals consumed a similar amount of control food.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Student's T test was used.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This study d	id not adequately measure the animals' intake	of DEHP, rendering it	unacceptable.			

Overall Quality Determination

Uninformative

Study Citation:	Peakall, D.	Peakall, D. B. (1974). Effects of di-n-butyl and di-2-ethylhexyl phthalate on the eggs of ring doves. Bulletin of Environmental Contamination and							
	Toxicology 1	Toxicology 12(6):698-702.							
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported								
Exposure Route,	ierrestriai; Food/Diet; Dietary								
Tava Species Age	Vertebrate: A	avian: Streptopelia risoria: Adult							
Health Outcome:	Developmen	t/Growth							
Chemical:	Di-ethylhexy	verowin vl phthalate (DEHP)							
HERO ID:	681729								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name.					
	Metric 2:	Test Substance Source	Low	The source was not reported and the test substance was not analytically verified by the performing laboratory.					
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	A concurrent negative control was included in the experiment.					
	Metric 5:	Negative Control Response	High	The biological responses of the negative control group were reported.					
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.					
Domain 3: Exposure Ch	naracterization								
	Metric 7:	Experimental System/Test Media Preparation	Uninformative	The testing methods were not described in the paper and the references cited in the methods are only for maintenance of cages (HERO ID: 3061674) and for outcome assessment methods. No information was given on how the diets were dosed with DEHP, feeding schedule, etc. Concentration of the test substance was not measured during the study.					
	Metric 8:	Consistency of Exposure	Low	Details of exposure administration were not reported.					
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.					
	Metric 10.	Concentration Exposure Duration and Frequency	Uninformative	The duration of exposure and exposure frequency were not reported					
	Metric 11:	Number of Exposure Groups/	Medium	Only one exposure group was used to assess eggshell thickness, weight, rate of water					
		Spacing of Exposure Levels		loss, surface area, and permeability.					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.					
Domain 4. Test Organis	m								
Domanii 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source was not reported.					
	Metric 14:	Acclimatization and Pretreatment	Low	The study does not report whether pretreatment conditions were the same for control					
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of dove pairs and replicates used were not reported. But it was reported that a total of 34 eggs were used to determine eggshell thickness index, weight, rate of water loss, surface area and permeability (Table 1).					

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Diethylhexyl Phthalate

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Study Citation:	Peakall, D.	Peakall, D. B. (1974). Effects of di-n-butyl and di-2-ethylhexyl phthalate on the eggs of ring doves. Bulletin of Environmental Contamination and						
D	Toxicology 1	Toxicology 12(6):698-702.						
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported							
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Avian; Streptopelia risoria; Adult						
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	681729							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Pairs of ring doves were maintained as described in Peakall, 1970. A feeding schedule was not provided.				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodologies were briefly described. References were cited for eggshell index calculation (Ratcliffe, D.A., 1970) and permeability determination (HERO ID: 2180519; pdf not available).				
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment (e.g.,				
		Assessment		timing of assessment across groups) were not reported.				
Domain & Confounding	Variable Co	ateal						
Domain 6: Confounding	g / Variable Col		т					
	Metric 19:	Confounding variables in Test	LOW	I he study did not provide enough information to allow a comparison of environmental				
	Matria 20.	Design and Procedures	Madium	There are a sinformation on extension are extension and the examples				
	Metric 20.	Outcomes Onrelated to Exposure	Ivieuluiii	There was no information on outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for the treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	ents: No details were given for the dietary toxicity testing methods. The duration of exposure and exposure frequency, and the number of pairs of doves used in							
	the experime	ent were not reported.						

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Ljungvall, K	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose						
	oral di(2ethy	(lhexyl) phthalate affects the peripheral LH-	concentration in J	plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive				
Dest	Toxicology 2	Toxicology $21(2):160-166$.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Terrestrial; I	food/Diet; Dietary						
Media, Path:	X 7 (1 ()		1. 0 1.11	1 411				
Taxa, Species, Age:	Vertebrate; I	Diammalian; Sus domesticus; Swedish York	snire x Swedish I	andrance; Adult				
Health Outcome:	Di athulhaw	-Biomarkers (exposure and effect)						
	682666	yi philialale (DEHP)						
HERO ID;	083000							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	Low	Correct nomenclature was reported, but no CASRN or figure of the structure were pro- vided.				
	Metric 2:	Test Substance Source	Low	The source of the chemical substance was listed as Sigma Aldrich, but the substance was not analytically verified.				
	Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.				
Domain 2: Test Design								
8	Metric 4:	Negative Controls	High	Control groups were administered with water.				
	Metric 5:	Negative Control Response	High	Control hormone responses were reported and adequate for every timepoint (Figure 2).				
	Metric 6:	Randomized Allocation	Medium	The authors reported random allocation of test animals to treatment groups.				
Domain 3: Exposure Ch	naracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods were described, but the preparation of the treat- ment concentrations did not detail the preparation of the oral gavage.				
	Metric 8:	Consistency of Exposure	High	Exposures were consistent among treatment and control groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual exposure concentration was reported.				
	Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was over four weeks when piglets were three weeks of age. This duration and exposure age was targeting the impacts of DEHP on the development of the HPG-axis (GnRH-stimulation). To examine the function of the HPG-axis, the boars were stimulated at the end of the recording period (nine months of age) with a GnRH analogue and the hormone concentrations in blood were measured.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form was administered via a gavage to pure DEHP.				
Domain 4: Test Organia	m							
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The source (experimental station at Lovsta, Gotland, Sweden) and strain (cross bred, Swedish Yorkshire × Swedish Landrace) were reported.				
		Conti	inued on next pa	ge				

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Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrance; Adult Mechanistic-Biomarkers (exposure and effect) Di-ethylhexyl phthalate (DEHP)				
Domain		Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment	High	Animal care was well described in section 2.1.	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	Eight DEHP-exposed and eight control boars were used for the GnRH-stimulation test (section 2.4). However, due to malfunctioning catheters, data from the GnRHstimulation post-stimulation was based on seven animals from each group (section 3.2 and Fig. 2). There were no replicate groups.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many care considerations are mirrored in 850.2400.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (GnRH-stimulation) addressed the intended outcome of interest (to examine DEHP impacts on the function of the HPG-axis) but representation (Fig. 2) of the assessment methodology should have been more clear. Furthermore, the authors reported catheter problems during the experiment, and thus, it is hard to interpret the one time-point display of significant differences in LH concentration between control and treatment groups at 30 minutes.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was reported and assessed consistently.	
Domain 6: Confounding	r / Variabla Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No environmental conditions were indicated that could alter treatment and control ani- mals.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost. There were also "sporadic cases of diarrhea" that were treated with IM-injections of trimethoprim-sulphonamide to the affected animals.	
Domain 7. Data Present	tation and Δnal	vsis			
Domain 7. Data Heseli	Metric 21:	Statistical Methods	Low	Authors indicated that parameters were analyzed using the Mixedprocedure of SAS (http://support.sas.com/). Data collected during the hour before the GnRH-stimulation were analyzed separately from the data after the GnRH-stimulation. Furthermore, paired t-tests were used to investigate the effects of DEHP on GnRH-stimulation. But authors did not report if adjustments were conducted for multiple statistical tests that were run (ie Bonferroni test/correction).	
	Metric 22:	Reporting of Data	High	Results were provided in Figure 2.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Problems with blood collection and catheters reduced the sample size for both treatment and control groups to seven individuals.	
		Contin	ued on next pa	ge	

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Diethylhexyl Phthalate

	•	continued from previous page					
Study Citation:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.						
Duration:	Overall Duration: > 21 days; Exposure Duration	n: > 21 days					
Exposure Route,	Terrestrial; Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Vertebrate; Mammalian; Sus domesticus; Swedi	Vertebrate; Mammalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Adult					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	683666						
Domain	Metric	Rating	Comments				
Additional Comments:	This form was used to evaluate the mechanistic i used to evaluate DEHP effects on plasma luteini	impacts of DEHP on the development of izing hormone and testosterone concentra	the HPG-axis (via GnRH-stimulation). Specifically, this form was ations after stimulation with GnRH in adult boars (Figure 2).				
Overall Qualit	ty Determination	Medium					

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Study Citation: Duration: Exposure Route, Media, Path:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary					
Health Outcome: Chemical:	Reproductive Di-ethylhexy	e/Teratogenic /I phthalate (DEHP)	ksnire x Swec	lish landrance; Adult		
Domain	000000	Metric	Rating	Comments		
Domain 1: Test Substan	ce		0			
	Metric 1:	Test Substance Identity	Low	Correct nomenclature was given, but no CASRN or figure of the structure were pro- vided.		
	Metric 2:	Test Substance Source	Low	The source of the chemical substance was listed as Sigma Aldrich, but the substance was not analytically verified.		
	Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Control groups were administered with water.		
	Metric 5:	Negative Control Response	High	Responses of the reproductive behavior endpoints were adequate for the control group (Tables 1, 2, and 3).		
	Metric 6:	Randomized Allocation	Medium	The authors report random allocation of test animals to treatment groups.		
Domain 3. Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods were described, but the preparation of the treat- ment concentrations did not detail the preparation of the oral gavage.		
	Metric 8:	Consistency of Exposure	High	Exposures were consistent among treatment and control groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual exposure concentration.		
	Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was 4 weeks when piglets were 3 weeks of age. This duration of exposure age was to examine the impacts of DEHP on mating behavior of male boars from 6 to 9 months of age.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form administered via a gavage to pure DEHP.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The source (experimental station at Lovsta, Gotland, Sweden) and strain (cross bred, Swedish Yorkshire × Swedish Landrace) were reported.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animal care was well described in section 2.1.		
Continued on next page						

Diethylhexyl Phthalate

Study Citation: Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-nata oral di(2ethylthexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pube Toxicology 21(2):160-166. Duration: Coverall Duration: > 21 days; Exposure Duration: > 21 days Exposure Route, Media, Path: Terrestrial; Food/Diet; Dietary Media, Path: Terrestrial; Food/Diet; Dietary Media, Path: Reproductive/Freatogenic Dimain Metric Rating Comments Domain Metric Rating Comments Domain 5: Outcome Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure proceducate of in 800-2000. Domain 5: Outcome Assessment Metric 17: Outcome Assessment Methodology High The authors adequately detailed care, handling, and exposure proceducate of in 800-2040. Domain 6: Confounding / Variable Control High No environmental conditions indicated that could after treatment and DEHP individual were encored and appropriate for the indeed outcome of in DEHP impacts on reproductive behavior). Domain 6: Confounding / Variable Control High The outcome assessment was reported and appropriate for the indetect anianalab, two exidence of on the study, wappropriate, Ter				nucu nom p	nethous page			
Duration: Overall Duration: > 21 days Exposure Duration: > 21 days Exposure Ronte, Exposure Ronte, Age: Terrestrial; Food/Diet; Dietary Media, Path: Taxa, Species, Age: Vertebrate: Mammalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Adult Health Outcome: Reproductive/Teratogenic Exposure Ronte, Comments Domain Metric 10: Reproductive/Teratogenic Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High High The authors adequately detailed care, handling, and exposure proceduce of rune methodology (mounting counts, time to more picaletary for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were used for the intended outcome of in DEHP individuals were removed from the study. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcome Superior Terestoria assessment were also "sporadic cases of diarthed" that were to in pictuals sporadic cases of diarthed" that were to in pictuals explaining if these sp	Study Citation:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dos oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductiv Toxicology 21(2):160-166.						
Exposure Route, Media, Path: Terrestrial; Food/Diet; Dietary Maxa, Species, Age: Vertebrate; Mammalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Adult Health Outcome: Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 683666 Domain Metric 15: Number of Organisms and Replicates per Group 8 control and 8 DEHP individuals were used for the mating behavior/ havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850-2400. Domain 6: Confounding / Variable Control Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mo ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures High Ne environmental conditions indicated that could alter treatment and Design and Procedures Mediaii The end of section 2.1 indicated that a control and treatment individua a result, the surving paired siblings were removed from the study, we are considerations of trimethoprim-sulphonamide to the affected animals, how details explaining if these sporadic cases diffecting the outcome sorganic cases of diai	Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Media, Path: Taxa, Species, Age: Vertebrate; Nammalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Adult Meath Outcome: Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 683666 Domain Metric Metric 15: Number of Organisms and Replicates per Group Reating Comments Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure proceducare considerations are mirrored in 850.2400. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure proceducare considerations are mirrored in 850.2400. Domain 5: Outcome Assessment Metric 16: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mot ergleutally was reported and appropriate for the intendo outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High No environmental conditions indicated that could alter treatment and Design and Procedures Domain 6: Confounding / Variable Contro- Metric 20: Confounding Variables in Test Design and Procedures High No environmental conditions indicated that could alter treatment and/ Design and Procedures Domain 7: Data Presentation and Analysis Metric 21:<	Exposure Route,	Terrestrial; F	Food/Diet; Dietary					
Taxa, Species, Age: Vertebrate: Marmalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Adult Health Outcome: Operatogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 683666 Comments Domain Metric 15: Number of Organisms and Replicates per Group Low 8 control and 8 DEHP individuals were used for the mating behavior/ havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment High The authors adequately detailed care, handling, and exposure proceds care considerations are mirrored in 850.2400. Metric 16: Adequacy of Test Conditions High The outcome assessment methodology (mounting counts, time to mot ejacutale) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment assessment and Design and Procedures Metric 20: Confounding Variables in Test Design and Procedures Metric 20: High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Domain 7: Data Presentation and Analysis Metric 18: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm genetiy of variance. Chi-square tests were conducted on reproductive matting behavior countain; Table 2:	Media, Path:							
Health Outcome: Reproductive/Teratogenic Chemical: Di-ethylhexyl pithalate (DEHP) HERO ID: 68366 Domain Metric 0 Reproductive/Teratogenic Metric Nomain Metric 15: Number of Organisms and Replicates per Group Low Replicates per Group Bomain 5: Outcome Assessment Metric 16: Metric 17: Outcome Assessment Methodology Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome Assessment Domain 6: Confounding / Variable Control High Assessment Domain 6: Confounding / Variable Control Metric 19: Outcome Support and Procedures Metric 20: Outcome Support and Procedures Metric 20: Domain 7: Data Presentation and Analysis Metric 21: Metric 21: Statistical Methods Metric 21: Statistical Methods Metric 21: Statistical Methods Metric 21: Statistical Methods	Taxa, Species, Age:	Vertebrate; N	Aammalian; Sus domesticus; Swedish York	shire x Swee	lish landrance; Adult			
Chemical: HERO ID: Di-ethylhexyl phthalate (DEHP) 683666 Domain Metric 15: Mumber of Organisms and Replicates per Group Low Association and 8 DEHP individuals were used for the mating behavior/ havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in \$50,2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mo ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on peroductive behavior). Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: High Outcomes Unrelated to Exposure No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: The end of section 2.1 indicated that a control and treatment individua aresult, the surviving paired siblings were removed from the study, wa appropriate. There were also "sporadic cases or of diarthea" that were trigictions of trimethoprim-sulphonamide to the affected animals, how defails explaining these sporadic cases or equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco maring behavior counts. Table 2: maning behavior duration; Table 3: 1 maning behavior counts. Table 2: maning behavior duration; Table 3: 1 maning behavior dur	Health Outcome:	Reproductive	e/Teratogenic					
HERO ID: 683666 Domain Metric Rating Comments Metric 15: Number of Organisms and Replicates per Group Low 8 control and 8 DEHP individuals were used for the mating behavior havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to more ipaculate) was reported and appropriate for the intended outcome of it DEHP impacts on reproductive behavior). Domain 6: Confounding / Variable Control High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures High Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were requely prevailed: naw ever injections of trimethorin-sulphonamide to the affected animals; how are used in explorabiling if these sporadic cases ever equally prevailent in the group or if there is evidence for the sporadic cases affecting the outco are used in the sporadic cases were equally prevailent in the group or if there is evidence for the sporadic cases were equally pr	Chemical:	Di-ethylhexy	l phthalate (DEHP)					
Domain Metric Rating Comments Metric 15: Number of Organisms and Replicates per Group Low 8 control and 8 DEHP individuals were used for the mating behavior/ havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mot ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome Assessment High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Domain 6: Confounding / Variable Control Metric 20: Confounding Variables in Test Design and Procedures Metric 20: High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: matin	HERO ID:	683666						
Metric 15: Number of Organisms and Replicates per Group Low 8 control and 8 DEHP individuals were used for the mating behavior havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mo ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome Assessment High Domain 6: Confounding / Variable Control Metric 20: Confounding Variables in Test Design and Procedures High Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that could alter treatment and Design and Procedures Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Metric 21: Statistical Methods High	Domain		Metric	Rating	Comments			
Replicates per Group havior experiments, which were conducted for 14 weeks (from 6-9 m There were no replicate groups. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure proceds care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to more ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that could alter treatment individua a result, the surviving paired siblings were removed from the study, wappropriate. There were also "sporadic cases of diarrhea" that were to injections of trimethoprim-subphonamide to the affected animals, the group or if there is evidence for the sporadic cases affecting the outcom easily appropriate. There were also "sporadic cases affecting the outcom easily appropriate. There were also "sporadic cases affecting the outcom easily appropriate. There were also "sporadic cases affecting the outcom easily a		Metric 15:	Number of Organisms and	Low	8 control and 8 DEHP individuals were used for the mating behavior/reproductive be-			
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mo or ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that could alter treatment and procedures Metric 20: Outcomes Unrelated to Exposure Medium There were also "sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outcome and splanage in the study, we appropriate. There were also "sporadic cases affecting the outcome appropriate. There were also "sporadic cases affecting the outcome are subphonamide to the affected aninals; how details explaining if these sporadic cases affecting the outcome are reported. Domain 7: Data Presentation and Analysis High Authors indicate "mixed procedure" in SAS after inspecting for norm genetity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: 1			Replicates per Group		havior experiments, which were conducted for 14 weeks (from 6-9 months of age).			
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to more jaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Assessment High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, wa appropriate. There were also "sporadic cases of diarrha" that were tringingctions of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting t					There were no replicate groups.			
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High The authors adequately detailed care, handling, and exposure procedu care considerations are mirrored in 850.2400. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to mon ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, wa appropriate. There were also "sporadic cases of diarrhea" that were tri injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcome group or if there is evidence for the sporadic cases affecting the outcom group or if there is evidence for the sporadic cases affecting the outcom genetity of variance. Chi-square tests were conducted on reproductive mating behavior duration; Table 3: 1	Domain 5: Outcome Ass	essment						
Metric 10. Adequacy of rest conductors Fight The autors adequately defined care, handling, and exposure procedure Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to more ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Assessment Assessment High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, wa appropriate. There were also "sporadic cases of diarrhea" that were tri injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcome or injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcome are equally procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior courts; Table 2: mating behavior duration; Table 3: 1	Johan J. Outcome Ass	Metric 16.	A dequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many			
Metric 17: Outcome Assessment Methodology High The outcome assessment methodology (mounting counts, time to more ejaculate) was reported and appropriate for the intended outcome of in DEHP impacts on reproductive behavior). Metric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Assessment High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, wa appropriate. There were also "sporadic cases of diarrhea" that were treating in these sporadic cases affecting the outcom details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting the outcom details explaining if these sporadic cases affecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: In the study of the sporadic case is the sporadic case.		Methe 10.	Adequacy of Test Conditions	Ingn	care considerations are mirrored in 850.2400.			
Metric 18: Consistency of Outcome High The outcome assessment was reported and appropriate for the intended outcome of i DEHP impacts on reproductive behavior). Domain 6: Confounding / Variable Control High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, wappropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco mere of the sporadic cases affecting the outco details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic case affecting the outco details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic case affecting the outco details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic case affecting the outco mere of the sporadic cases were equally prevalent in the group or if there is evidence for the sporadic case affecting the outco mere of the sporadic case were equally prevalent in the group or if there is evidence for the sporadic cases aff		Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mounting counts, time to mount, attempts to			
Detric 18: Consistency of Outcome High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tri nijections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcom Domain 7: Data Presentation and Analysis High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior duration; Table 3: 1				e	ejaculate) was reported and appropriate for the intended outcome of interest (to examine			
Metric 18: Consistency of Outcome Assessment High The outcome assessment was reported and assessed consistently. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures High No environmental conditions indicated that could alter treatment and result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: I					DEHP impacts on reproductive behavior).			
Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individu: a result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis High Metric 21: Statistical Methods		Metric 18:	Consistency of Outcome	High	The outcome assessment was reported and assessed consistently.			
Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures High No environmental conditions indicated that could alter treatment and Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, we appropriate. There were also "sporadic cases of diarrhea" that were treinjections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases affecting the outcomes of the sporadic c			Assessment					
Metric 19: Confounding Variables in Test Design and Procedures High No environmental conditions indicated that could alter treatment and Metric 20: Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individu: a result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: High	Domain 6: Confounding	/ Variable Cor	atrol					
Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individua a result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H		Metric 19:	Confounding Variables in Test	High	No environmental conditions indicated that could alter treatment and control animals.			
Metric 20: Outcomes Unrelated to Exposure Medium The end of section 2.1 indicated that a control and treatment individu a result, the surviving paired siblings were removed from the study, w appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H			Design and Procedures	i iigii				
appropriate. There were also "sporadic cases of diarrhea" that were tr injections of trimethoprim-sulphonamide to the affected animals; how details explaining if these sporadic cases were equally prevalent in the group or if there is evidence for the sporadic cases affecting the outco Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H		Metric 20:	Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost; as a result, the surviving paired siblings were removed from the study, which was most			
Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H					appropriate. There were also "sporadic cases of diarrhea" that were treated with IM-			
Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: High					injections of trimethoprim-sulphonamide to the affected animals; however, there were no			
Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H					details explaining if these sporadic cases were equally prevalent in the control or DEHP			
Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: H					group or if there is evidence for the sporadic cases affecting the outcomes of interest.			
Metric 21: Statistical Methods High Authors indicate "mixed procedure" in SAS after inspecting for norm geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: I	Domain 7: Data Presenta	ation and Anal	vsis					
geneity of variance. Chi-square tests were conducted on reproductive mating behavior counts; Table 2: mating behavior duration; Table 3: I		Metric 21:	Statistical Methods	High	Authors indicate "mixed procedure" in SAS after inspecting for normality and homo-			
& gigeulation)				6	geneity of variance. Chi-square tests were conducted on reproductive behavior (Table 1: mating behavior counts; Table 2: mating behavior duration; Table 3: Percentage mounts			
Metric 22: Reporting of Data High All behavior data are presented within Tables 1-3		Metric 22.	Reporting of Data	High	All behavior data are presented within Tables 1-3			
Metric 22. Explanation of Unexpected Outcomes High No unexpected outcomes were noted by the outbors and measure of u		Metric 22.	Explanation of Unexpected Outcomes	High	No unexpected outcomes were noted by the authors and measure of variability (SE) was			
provided.		wienie 23.	Explanation of Olicypected Outcomes	mgn	provided.			

Overall Quality Determination

High

Study Citation:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166						
Duration:	Overall Duration: > 21 days: Exposure Duration: > 21 days						
Exposure Route.	Terrestrial: H	Food/Diet: Dietary	1 days				
Media. Path:							
Taxa, Species, Age:	Vertebrate: N	Mammalian: Sus domesticus: Swedish Yor	kshire x Swea	lish landrance: Juvenile			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	683666	()					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Correct nomenclature was reported, but no CASRN or figure of the structure were pro- vided.			
	Metric 2:	Test Substance Source	Low	Source was listed as Sigma Aldrich but was not analytically verified.			
	Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4.	Negative Controls	High	Control groups were administered with water			
	Metric 5:	Negative Control Response	High	The biological response (plasma concentrations of LH, oestradiol and testosterone at			
				30 minute intervals for 6 hours) before and during the 4 weeks of repeated (three times weekly) administration of 300 mg/kg DEHP was reported and adequate for the control group (Fig. 1).			
	Metric 6:	Randomized Allocation	Medium	The authors report random allocation of test animals to treatment groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Medium	The experimental system and methods were described, but the preparation of the treat-			
	Metrie 7.	Preparation	Wiedium	ment concentrations did not detail the preparation of the oral gavage.			
	Metric 8:	Consistency of Exposure	High	Exposures were consistent among treatment and control groups.			
		Administration	0				
	Metric 9:	Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual concentration.			
	Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was over 4 weeks when piglets were 3 weeks of age. This duration and exposure age was adequate for the study type (the impacts of DEHP, endocrine disrupter, on the development of the HPG-axis).			
	Metric 11:	Number of Exposure Groups/	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure			
		Spacing of Exposure Levels		concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form administered via a gavage to pure DEHP.			
Domain 4: Test Organis	III Matria 12:	Test Organism Characteristic-	High	The same (an end at the start of the start Caller & Causton) and staring (
	Metric 13:	rest Organism Unaracteristics	High	Swedish Yorkshire × Swedish Landrace) were reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animal care and pretreatment conditions were well described in section 2.1.			
		Cont	inued on nex				

Diethylhexyl Phthalate

		conti	nued from p	previous page			
Study Citation: Duration: Exposure Route, Media Path:	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Vertebrate; N	Mammalian: Sus domesticus; Swedish York	shire x Swee	lish landrance: Juvenile			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	683666						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Plasma concentrations of LH, oestradiol and testosterone (Fig. 1): authors reported us- ing 20 individual animals per group (DEHP or placebo (water). There were no replicate groups.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many care considerations are mirrored in 850.2400.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (radioimmunoassay to measure plasma hormone concentrations) was reported and adequate for the intended outcome of interest (impacts of DEHP on concentrations of reproductive hormones in plasma).			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was reported and assessed consistently.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No environmental conditions indicated that could alter treatment and control animals.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost; as a result, the surviving paired siblings were removed from the study, which was most appropriate. There were also "sporadic cases of diarrhea" that were treated with IM-injections of trimethoprim-sulphonamide to the affected animals; however, there were no details explaining if these sporadic cases were equally prevalent in the control or DEHP group or if there is evidence for the sporadic case affecting the outcomes of interest.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Authors indicate "mixed procedure" in SAS after inspecting for normality and homo- geneity of variance. All data used in the Mixed procedure were log-transformed to im- prove the normal distribution and the homogeneity of variances. The least square option was used to compare different means at different time-points for the hormonal data.			
	Metric 22:	Reporting of Data	High	Figure 1 presents exposure related findings adequately.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were noted by the authors and measure of variability (SE) was provided.			
Additional Comments:	This form w during the 4	as used to evaluate the impacts of DEHP week exposure of DEHP (Figure 1).	on sex horm	one (testosterone, oestradiol 17-beta, and LH) concentrations in the plasma in piglets			

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166. **Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days **Exposure Route**, Terrestrial; Food/Diet; Dietary Media, Path: Vertebrate; Mammalian; Sus domesticus; Swedish Yorkshire x Swedish landrance; Juvenile Taxa, Species, Age: Health Outcome: Mechanistic-Biomarkers (exposure and effect) Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 683666 Metric Domain Rating Comments **Overall Quality Determination** High

Study Citation: Duration: Exposure Route, Media, Path:	Spjuth, L., I phthalate in Overall Dura Terrestrial; F	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; N Reproductive Di-ethylhexy 683808	Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshir e/Teratogenic yl phthalate (DEHP)	e x Swedish I	andrace; Juvenile			
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by nomenclature but structure and CASRN were not provided.			
	Metric 2:	Test Substance Source	High	Obtained from Sigma-Aldrich, Stockholm, Sweden.			
	Metric 3:	Test Substance Purity	High	99.5% purity.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	One sibling from each of ten pairs was randomly assigned to control.			
	Metric 5:	Negative Control Response	High	One mortality in negative controls, from endocarditis (resulting in 10% mortality rate). There was one mortality in DEHP treated group as well, from myositis. The corresponding sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the experiment was therefore 16 (eight in each group).			
	Metric 6:	Randomized Allocation	Medium	Explicitly stated that one pig from each pair, with pairs taken from 10 separate litters, was randomly assigned to DEHP or control group.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	Pure DEHP was administered orally via blunt syringe.			
	Metric 8:	Consistency of Exposure Administration	High	Administration was the same for all groups, including control (i.e., placebo administra- tion of same amount of water).			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Dietary exposure was to the pure compound via blunt syringe, but the DEHP dose of 300 mg/kg (i.e. 0.3 mL/kg) body weight was not measured/analytically verified.			
	Metric 10:	Exposure Duration and Frequency	High	Exposure thrice weekly from 3 weeks of age to 7 weeks of age (1 month exposure) in pre-pubertal boars was appropriate to examine the effects of DEHP exposure on sperm quality later in life (up to 9 months of age).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to determine a dose-response relationship.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was oral administration of the pure compound.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Piglets were obtained from research station stock and reared at the Swedish University of Agricultural Sciences, Uppsala, Sweden.			
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Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route, Media, Path:	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; N Reproductive Di-ethylhexy 683808	Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshir e/Teratogenic rl phthalate (DEHP)	re x Swedish I	.andrace; Juvenile		
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all piglets were weaned at 3 weeks postpartum and from then until 5 months of age were housed in two boxes, one for each group (DEHP or control). Also, they reported that one month before collection of semen started, i.e. at five months of age, the animals were moved to individual pens with straw bedding at the Division of Comparative Reproduction, Obstetrics and Udder Health, SLU, Uppsala, Sweden.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 piglets were used in each group. Until 5 months of age, all piglets were housed in two boxes, one for each group(DEHP or control). At 5 months of age, the animals were moved to individual pens.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Details of husbandry conditions were minimal (temperature, feed amount and frequency, pen size were not reported), but there are no details in the study to indicate that conditions would have an impact on results.		
	Metric 17:	Outcome Assessment Methodology	High	The methodologies to evaluate ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity were appropriate to examine sperm quality impacts later in life following exposure during pre-puberty). The methodologies employed for sperm quality examination were appropriate and well-detailed.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.		
Domain 6: Confounding	v / Variable Cou	atrol				
Domani 0. Comountaing	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was one mortality in each group of control and treatment from endocarditis and myositis, respectively; for these two pigs, the corresponding paired sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the study was 16 (8 per group). Also, there were sporadic cases of diarrhea that was treated with intramuscular trimethprim-sulfonamide if animal condition was affected, but no details on were reported on whether controls or treatment animals were more affected.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21: Metric 22:	Statistical Methods Reporting of Data	High High	Statistical methods were clearly described (nested model run in SAS). Data for treatment & control presented.		

Diethylhexyl Phthalate

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Study Citation:	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty? International Journal of Andrology 29(5):534-542				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Terrestrial; Food/Diet; Dietary				
Media, Path:					
Taxa, Species, Age:	Vertebrate; M	ammalian; Sus scrofa; Swedish Yorkshire	x Swedish I	Landrace; Juvenile	
Health Outcome:	Reproductive/Teratogenic				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	683808				
Domain		Metric	Rating	Comments	
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes (DEHP-exposed animals had slightly lower rates of spermatozoa morphological abnormalities than controls) were thoroughly explained (small effect size that disappeared by the time boars were 8 months old, possible artifact of subjective sperm quality assays).	
Additional Comments: This study examined the semen and sperm quality effects of of orally-administered pure DEHP (300 mg/kg bw) to domestic boars several months after exposure. A comprehensive suite of semen and sperm quality parameters was evaluated in a high-quality lab that serves as the reference laboratory for semen evaluation in Sweden. Assessed semen parameters included ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity. All findings have been evaluated in this form because all parameters were examined in detail and no negative effects of early oral exposure to DEHP on the post-pubertal sperm output and sperm quality were found.					
Overall Qualit	y Determ	nination	High		

HERO ID: 683808 Table: 2 of 2

Study Citation:	Spjuth, L., 1	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl)					
Duration: Exposure Route, Media. Path:	phthalate in Overall Dura Terrestrial; I	Derrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; I Mechanistic Di-ethylhex	Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshir -Cytotoxicity yl phthalate (DEHP)	e x Swedish I	Landrace; Juvenile			
Domain	083808	Metric	Pating	Comments			
Domain 1: Test Substan	ce.	Metric	Katilig	Comments			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by nomenclature but structure and CASRN were not pro- vided.			
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, Stockholm, Sweden.			
	Metric 3:	Test Substance Purity	High	The purity was reported as 99.5%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	One sibling from each of ten pairs was randomly assigned to the control.			
	Metric 5:	Negative Control Response	High	There was one mortality in the negative controls, resulting from endocarditis (resulting in 10% mortality rate). There was one mortality in DEHP treated group as well, resulting from myositis. The corresponding sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the experiment was therefore 16 (eight in each group).			
	Metric 6:	Randomized Allocation	Medium	It was explicitly stated that one pig from each pair, with pairs taken from 10 separate litters, was randomly assigned to DEHP or the control group.			
Domain 2. Expansion Ch	anastanization						
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media	High	Dura DEHD was administered orally via blunt swinge			
	Wieute 7.	Preparation	Ingn	Tute DETT was auministered orany via bluit symige.			
	Metric 8:	Consistency of Exposure Administration	High	Administration was the same for all groups, including control (i.e., placebo administra- tion of same amount of water).			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Dietary exposure was to the pure compound via blunt syringe, but the DEHP dose of 300 mg/kg (i.e. 0.3 mL/kg) body weight was not measured/analytically verified.			
	Metric 10:	Exposure Duration and Frequency	High	Exposure was thrice weekly from three weeks of age to seven weeks of age (one-month exposure) in pre-pubertal boars was appropriate to examine the effects of DEHP exposure on sperm quality later in life (up to nine months of age).			
	Metric 11:	Number of Exposure Groups/	N/A	The study goal was not to determine a dose-response relationship.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was oral administration of the pure compound.			
Domain 4: Test Organis	m Metric 13:	Test Organism Characteristics	High	Piglets were obtained from research station stock and reared at the Swedish University of Agricultural Sciences, Uppsala, Sweden.			
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Diethylhexyl Phthalate

	continued from previous page						
Study Citation:	Spjuth, L., I	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl)					
	phthalate in	phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial: Food/Diet: Dietary					
Exposure Route,	Terrestrial; F	food/Diet; Dietary					
Media, Path:		Vartahrata: Mammalian: Sus sarafa: Swadish Varkshira x Swadish Landraca: Juvanila					
Taxa, Species, Age:	Machanistia	Vertebrate; Mammalian; Sus scroja; Swedish Yorkshire x Swedish Landrace; Juvenile					
Chamical:	Di athylhayyl phthalata (DEHD)						
HERO ID.	683808	(DEIII)					
	000000		D.:				
Domain	Matria 14	Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all piglets were weaned at three weeks postpartum and from then until five months of age were housed in two boxes, one for each group (DEHP or con- trol). Also, they reported that one month before collection of semen started, i.e. at five months of age, the animals were moved to individual pens with straw bedding at the Division of Comparative Reproduction, Obstetrics and Udder Health, SLU, Uppsala, Sweden.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Ten piglets were used in each group. Until five months of age, all piglets were housed in two boxes, one for each group(DEHP or control). At five months of age, the animals were moved to individual pens.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16.	Adequacy of Test Conditions	Medium	Details of husbandry conditions were minimal (temperature feed amount and frequency			
	incure ro.	recently of rost conditions		pen size were not reported), but there are no details in the study to indicate that condi- tions would have an impact on results.			
	Metric 17:	Outcome Assessment Methodology	High	The methodologies to evaluate ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity were appropriate to examine sperm quality impacts later in life following exposure during pre-puberty). The methodologies employed for sperm quality examination were appropriate and well-detailed.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across groups.			
		Assessment					
Domain 6: Confounding	y / Variable Cou	ntrol					
2 onium 0. Comounding	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures	8	or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was one mortality in each group of control and treatment from endocarditis and myositis, respectively; for these two pigs, the corresponding paired sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the study was 16 (8 per group). Also, there were sporadic cases of diarrhea that were treated with intramuscular trimethprim-sulfonamide if animal condition was affected, but no details were reported on whether controls or treatment animals were more affected.			
Domain 7: Data Present	ation and Anal	vsis					
2 chiuni / Duu 11050m	Metric 21:	Statistical Methods	High	Statistical methods were clearly described (nested model run in SAS).			
	Metric 22:	Reporting of Data	High	Data for treatment & control groups was presented.			

Continued on next page ...

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Diethylhexyl Phthalate

	contin	ued from p	previous page		
Study Citation:	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty? International Journal of Andrology 29(5):534-542				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Terrestrial; Food/Diet; Dietary				
Media, Path:					
Taxa, Species, Age:	Vertebrate; Mammalian; Sus scrofa; Swedish Yorkshire	x Swedish I	Landrace; Juvenile		
Health Outcome:	Mechanistic-Cytotoxicity				
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	683808				
Domain	Metric	Rating	Comments		
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes (DEHP-exposed animals had slightly lower rates of spermatozoa morphological abnormalities than controls) were thoroughly explained (small effect size that disappeared by the time boars were eight months old, possible artifact of subjective sperm quality assays).		
Additional Comments:	dditional Comments: This study examined the semen and sperm quality effects of orally-administered pure DEHP (300 mg/kg bw) to domestic boars several months after exposure. A comprehensive suite of semen and sperm quality parameters was evaluated in a high-quality lab that serves as the reference laboratory for semen evaluation in Sweden. Assessed semen parameters included ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity. All findings have been evaluated in this form because all parameters were examined in detail and no negative effects of early oral exposure to DEHP on the post-pubertal sperm output and sperm quality were found. This form is for the mechanistic outcome of sperm plasma membrane integrity.				
Overall Qualit	ty Determination	High			

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How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Invantahuata	Invertebrate: Worms (e.g. Annelids, Nematodes): <i>Caenorhabditis elegans</i> : Wild Type N2: Larvae						
Invertebrate;	averiedraie; worms (e.g., Annends, Nematodes); Caenorhabaitis elegans; Wild Type N2; Larvae Reproductive/Teratogenic						
Di-ethylbeyy	Di-ethylbexyl phthalate (DEHP)						
5593882	i philialate (DEIII)						
	Metric	Rating	Comments				
ce							
Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.				
Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.				
Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.				
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
aracterization		Ŧ					
Metric /:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."				
Metric 8:	Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S- basal medium containing E. coli OP50 at 20 C for 72 h to reach adult stage."				
Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (reproduc- tion/development).				
Metric 11:	Number of Exposure Groups/	Medium	There were minor limitations regarding the number of exposure groups (2).				
	Spacing of Exposure Levels						
Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.				
n							
Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source				
Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms				
	Conditions						
Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient.				
	with insulin/ Overall Dura Terrestrial; C Invertebrate; Reproductive Di-ethylhexy 5593882 Retric 1: Metric 2: Metric 2: Metric 3: Metric 4: Metric 5: Metric 5: Metric 6: aracterization Metric 6: Metric 8: Metric 8: Metric 9: Metric 10: Metric 11: Metric 12: Metric 13: Metric 14: Metric 15:	with insulin/IGF-1-like signaling pathway and SKN-1 ir Overall Duration: 0 - 4 days (0-96h); Exposure Duration Terrestrial; Cell Culture Media; Not determined by study Invertebrate; Worms (e.g., Annelids, Nematodes); Caena Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 5593882 <u>Metric 1:</u> Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation aracterization Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Test Organism Characteristics Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Number of Organisms and Replicates per Group	with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-9 Terrestrial; Cell Culture Media; Not determined by study authors (i.e., ch Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegan Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 5593882 <u>Metric 1:</u> Test Substance Identity Metric 2: Test Substance Identity Metric 3: Test Substance Source Low Metric 3: Test Substance Purity Low Metric 4: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Metric 9: Measurement of Test Substance Low Administration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Metric 12: Testing at or Below Solubility Limit Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Metric 14: Acclimatization and Pretreatment Metric 14: Acclimatization and Pretreatment Metric 15: Number of Organisms and Metric 16: Number of Organisms and Metric 15: Number of Organisms and Medium Replicates per Group				

Diethylhexyl Phthalate

		contir	nued from previo	us page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	How, C. M., with insulin/ Overall Dur: Terrestrial; G Invertebrate: Reproductiv Di-ethylhex	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae Reproductive/Teratogenic				
HERO ID:	5593882					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health.		
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$. Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	This evaluat	ion is for brood size.				
Overall Qualit	ty Deterr	nination	Medium			

Study Citation: Duration: Exposure Route, Media, Path:	How, C. M., with insulin/ Overall Dura Terrestrial; C	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic Di-ethylhexy 5593882	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology) Di-ethylhexyl phthalate (DEHP) 5593882					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce Metric 1: Metric 2:	Test Substance Identity	Low Low	Chemical was identified by name only The test substance identity was not analytically verified by the performing laboratory			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	Maracterization Metric 7: Metric 8: Metric 9: Metric 10: Metric 11: Metric 12:	Experimental System/Test Media Preparation Consistency of Exposure Administration Measurement of Test Substance Concentration Exposure Duration and Frequency Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit	Low Low Low High N/A High	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control." Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S- basal medium containing E. coli OP50 at 20 C for 72 h to reach adult stage." Exposure concentrations were not measured The duration of exposure was reported and appropriate for the study type (biomarkers) Only one concentration tested A solvent was used to enhance the solubility of DEHP.			
			6				
Domain 4: Test Organis	m						
	Metric 13: Metric 14:	Test Organism Characteristics Acclimatization and Pretreatment	High High	The test organisms were adequately described and were obtained from a reliable source. all pretreatment conditions were the same for control and exposed organisms			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient			
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental conditions of test system were conducive to maintenance of organism health							
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 5593882 Table: 2 of 4

		contin	ued from previ	ious page			
Study Citation:	How, C. M., with insulin/	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	; Worms (e.g., Annelids, Nematodes); <i>Caeno</i>	rhabditis elega	ns; Wild Type N2; Larvae			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Oxidative	stress (includii	ng redox biology)			
Chemical: HERO ID:	Di-ethylhexy 5593882	yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at p < 0.05. Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate			
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained			
Additional Comments:	intracellular	chemicals and mRNA analysis	mgn	unexpected outcomes were satisfactoriny explained			

Overall Quality Determination

Medium

Study Citation: Duration:	How, C. M., with insulin/ Overall Dura	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Modia Dath:	Terrestriar, cen culture media, not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact up							
Tava Species Age	Invertebrate	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae Nutritional & Metabolic						
Health Outcome	Nutritional							
Chemical:	Di-ethylbex	i-ethylhexyl phthalate (DEHP)						
HERO ID:	5593882	(2211)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization		-					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."				
	Metric 8:	Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S- basal medium containing E. coli OP50 at 20 C for 72 h to reach adult stage."				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (metabolic response)				
	Metric 11:	Number of Exposure Groups/	N/A	The solvent concentration was appropriate (0.1% DMSO)				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.				
Domain 4: Test Organic	m							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source				
	Metric 14	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms				
	MCult 17.	Conditions	111511	an preteaution continuous were the same for control and exposed organisms				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient				
Domain 5: Outcome As	sessment							

Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	How, C. M., with insulin/	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caeno	orhabditis elegai	ns; Wild Type N2; Larvae			
Health Outcome:	Nutritional &	2 Metabolic	0				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5593882						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
Domain 6: Confounding	g / Variable Con Metric 19:	ntrol Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$. Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate			
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained			
Additional Comments:	defecation cy	ycle, pharyngeal pump					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route	How, C. M., with insulin/ Overall Dura Terrestrial: (How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial: Cell Culture Media: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route).				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Behavioral Di-ethylhexy	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae Behavioral Di-ethylhexyl phthalate (DEHP)				
HERO ID:	5593882					
Domain	22	Metric	Rating	Comments		
Domain 1. Test Substan	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	Low Low Low	Chemical was identified by name only The test substance identity was not analytically verified by the performing laboratory. Purity or grade of test substance were not reported		
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
	, · ,·					
Domain 3: Exposure Cn	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was0.1% as solvent control."		
	Metric 8:	Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S-basal medium containing E. coli OP50 at 20 C for 72 h to reach adult stage."		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10: Metric 11:	Concentration Exposure Duration and Frequency Number of Exposure Groups/	High Medium	The duration of exposure was reported and appropriate for the study type (behavioral) There were minor limitations regarding the number of exposure groups, all had signifi- cant effects		
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.		
Domain 4: Test Organis	m			-		
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient		
Domain 5: Outcome Ac	recoment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health		
	Continued on next page					

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Diethylhexyl Phthalate

		continu	ed from previ	ious page			
Study Citation: Duration: Exposure Route, Media, Path:	How, C. M., with insulin/ Overall Dura Terrestrial; C	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caeno	rhabditis elega	ns; Wild Type N2; Larvae			
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5593882						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups			
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$. Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate			
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained			
Additional Comments:	behavior-ber	behavior-bends and thrashes					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics				
HERO ID:	5555457	I phinalale (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substance	ce				
	Metric 1:	Test Substance Identity	Low	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."	
	Metric 2:	Test Substance Source	Low	DEHP was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). It was not analytically verified prior to test initiation.	
	Metric 3:	Test Substance Purity	Low	Purity and grade were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative solvent control (1% DMSO) was used.	
	Metric 5:	Negative Control Response	High	Control responses were adequate for the test.	
	Metric 6:	Randomized Allocation	Low	The allocation procedure was not reported.	
Domain 3: Exposure Cha	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The concentration was not measured during the study, nor was there any discussion on how loss of test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.	
	Metric 8:	Consistency of Exposure	High	Exposures were consistent for all groups.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Matric 10:	Concentration Exposure Duration and Frequency	High	Exposure duration was appropriate for the test	
	Metric 11	Number of Exposure Groups/	High	The number of exposure groups and spacing were adequate for the test. "Synchronized	
	metric 11.	Spacing of Exposure Levels	mgn	wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP $(0.2, 2, 20, 100 \text{ mg/L})$ or 0.1% DMSO (solvent control) and fed with E. coli OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."	
	Metric 12:	Testing at or Below Solubility Limit	High	DEHP was added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.	

Domain 4: Test Organism

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Environmental Hazard Evaluation

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HERO ID: 5555457 Table: 1 of 3

Study Citation:	Li, S. W., H elegans. Scie	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266. Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics Di-ethylhexyl phthalate (DEHP) 5555457							
Domain		Metric	Rating	Comments				
	Metric 13:	Test Organism Characteristics	High	The organisms used were hermaphroditic. Their life stage and age were well described. "Wild-type N2 C. elegans was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L cholesterol) that were seeded with a lawn of Escherichia coli OP50 as a food source in a 20 °C incubator. Synchronization of C. elegans was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchronized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S-basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to the test substance.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	Each test group contained at least five worms. Replicates were not reported.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate for the test.				
	Metric 17:	Outcome Assessment Methodology	High	The intended outcome (reproduction) was reported.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.				
Domain 6: Confounding	g / Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.				
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	High	Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean \pm standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05."				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported.				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.				
Continued on next page								

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Diethylhexyl Phthalate

	continued from previous page					
Study Citation:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but un	able to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	: Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae				
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5555457					
Domain	Metric Rating Comment	is				
Additional Comments:	ts: For Metric 7. Experimental System/Test Media Preparation: Nominal concentrations only were reported from the onset of the test. Given the hydrophobic nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable. This form is for the mechanistic outcomes reported in Fig. 6 for the F0 generation to the F5 generation.					

Overall Quality Determination

High

Study Citation:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis					
	elegans. Science of the Total Environment 634	4:260-266.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure	e Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; Cell Culture Media; Not determine	ed by study authors (i.e., chemical of inte	erest in exposure water, but unable to determine exact uptake route)			
Media, Path:						
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematod	les); Caenorhabditis elegans; Wild-type 1	N2; Larvae			
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5555457					
Domain	Metric	Pating	Comments			

Domain		Metric	Rating	Comments
Domain 1: Test Subst	ance			
	Metric 1:	Test Substance Identity	Low	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."
	Metric 2:	Test Substance Source	Low	DEHP purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). It was no analytically verified prior to test initiation.
	Metric 3:	Test Substance Purity	Low	Purity and grade were not reported.
Domain 2: Test Desig	n			
c	Metric 4:	Negative Controls	High	Negative solvent control (1% DMSO) used.
	Metric 5:	Negative Control Response	High	Control response were adequate for the test.
	Metric 6:	Randomized Allocation	Low	Allocation procedure not reported.
Domain 3: Exposure	Characterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	Concentration not measured during study, nor was there any discussion on how loss test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.
	Metric 8:	Consistency of Exposure	High	Exposures were consistent for all groups.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing were adequate for the test. "Synchronized wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with E. coli OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."
	Metric 12:	Testing at or Below Solubility Limit	High	DEHP added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.

Domain 4: Test Organism

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Environmental Hazard Evaluation

HERO ID: 5555457 Table: 2 of 3

continued from previous page							
Study Citation:	Li, S. W., H elegans, Scie	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	y authors (i.e., cl	hemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caena	orhabditis elega	ns; Wild-type N2; Larvae			
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5555457						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	High	Organisms are hermaphrodite, life stage and age well described. "Wild-type N2 C. ele- gans was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L choles- terol) that were seeded with a lawn of Escherichia coli OP50 as a food source in a 20 °C incubator. Synchronization of C. elegans was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchro- nized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S- basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to test substance.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Each test group contained at least 5 worms. Replicates were not reported.			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate for test.			
	Metric 17:	Outcome Assessment Methodology	High	Intended outcome (reproduction) was reported.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.			
Domain 6: Confoundin	g / Variable Co	ntrol					

Domain 7: Data Presentation and Analysis Metric 21: Statistical Me

Metric 19:

Metric 20:

Confounding Variables in Test

Outcomes Unrelated to Exposure

Design and Procedures

Statistical Methods High Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean \pm standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05." Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported. Metric 23: Explanation of Unexpected Outcomes High No unexpected outcomes were reported.

No confounding variables were reported.

No outcomes unrelated to exposure were reported.

High

High

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

		continued from previous page				
Study Citation:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis					
	elegans. Science of the Total Environment 6.	34:260-266.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	ure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; Cell Culture Media; Not determine	ned by study authors (i.e., chemical of inter	est in exposure water, but unable to determine exact uptake route)			
Media, Path:						
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nemato	odes); Caenorhabditis elegans; Wild-type N	2; Larvae			
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5555457					
Domain	Metric	Rating	Comments			
Additional Comments:	For Metric 7. Experimental System/Test Me	dia Preparation: Nominal concentrations on	ly were reported from the onset of the test. Given the hydrophobic			
	nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable.					
		· · · · ·	-			

Overall Quality Determination

Medium

Study Citation:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caend	orhabditis elegai	<i>is</i> ; Wild-type N2; Larvae			
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	5555457						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."			
	Metric 2:	Test Substance Source	High	DEHP purchased from Sigma-AldrichChemicals Co. (St. Louis, MO, USA)			
	Metric 3:	Test Substance Purity	Low	Purity and grade were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative solvent control (1% DMSO) used.			
	Metric 5:	Negative Control Response	High	Control response were adequate for the test.			
	Metric 6:	Randomized Allocation	Low	Allocation procedure not reported.			
Domain 3: Exposure Cha	aracterization						
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Low	Concentration not measured during study, nor was there any discussion on how loss test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.			
	Metric 8:	Consistency of Exposure	High	Exposures were consistent for all groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was appropriate for test.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing were adequate for the test. "Synchronized			
		Spacing of Exposure Levels	0	wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with E. coli OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."			
	Metric 12:	Testing at or Below Solubility Limit	High	DEHP added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.			

Domain 4: Test Organism

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Environmental Hazard Evaluation

HERO ID: 5555457 Table: 3 of 3

		cor	tinued from previ	ous page		
Study Citation:	Li, S. W., H	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis				
Duration	Overall Dur	ence of the Total Environment $6.34:260-2$	66.)6h)		
Exposure Route.	Terrestrial: (Cell Culture Media: Not determined by st	udv authors (i.e., cl	nemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	, -					
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Ca	enorhabditis elega	ns; Wild-type N2; Larvae		
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5555457					
Domain		Metric	Rating	Comments		
	Metric 13:	Test Organism Characteristics	High	Organisms are hermaphrodite, life stage and age well described. "Wild-type N2 C. ele- gans was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L choles- terol) that were seeded with a lawn of Escherichia coli OP50 as a food source in a 20 °C incubator. Synchronization of C. elegans was achieved by treating gravid hermaphrodite		

			nematodes with bleaching medium (0.45 M NaOH, 2% HOCI) to collect the synchro- nized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S- basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."
Metric	14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to test substance.
Metric	15: Number of Organisms and Replicates per Group	Low	Each test group contained at least 20 worms. Replicates were not reported.
Domain 5: Outcome Assessment			
Metric	16: Adequacy of Test Conditions	High	Environmental conditions were adequate for test.
Metric	17: Outcome Assessment Methodology	High	Intended outcome (behavior) was reported.
Metric	18: Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.
Domain 6: Confounding / Variab	le Control		
Metric	19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
Metric	20: Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and	Analysis		
Metric	21: Statistical Methods	High	Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean \pm standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05."
Metric	22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported.
Metric	23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5555457 Table: 3 of 3

		continued from previous page				
Study Citation:	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	re Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; Cell Culture Media; Not determin	ned by study authors (i.e., chemical of inter-	est in exposure water, but unable to determine exact uptake route)			
Media, Path:						
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nemato	des); Caenorhabditis elegans; Wild-type N	2; Larvae			
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5555457					
Domain	Metric	Rating	Comments			
Additional Comments:	For Metric 7. Experimental System/Test Med	lia Preparation: Nominal concentrations on	ly were reported from the onset of the test. Given the hydrophobic			
	nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable.					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae Nutritional & Metabolic Di-ethylhexyl phthalate (DEHP) 4728405				
Domain		Metric	Rating	Comments	
Domain 1: Test Substance	ce				
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.	
	Metric 2:	Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.	
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) listed at 99.5%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.	
	Metric 5:	Negative Control Response	High	Figure 3 (page 5/8) lists the control responses for fat staining are presented with color intensity and body area.	
	Metric 6:	Randomized Allocation	Low	No random allocation was described.	
Domain 3: Exposure Cha	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.	
	Metric 8:	Consistency of Exposure Administration	High	The two treatment concentrations appear to be administered similarly.	
	Metric 9:	Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, concentrations are reported as nominal.	
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr duration for this fat staining bioassay appear to be appropriate for C. elegans.	
	Metric 11:	Number of Exposure Groups/	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	value for this species. The growth media for C elegans is a solid	
			1.11.1		
Domain 4: Test Organism	n				
	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).	
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration were not reported in the methods but are available in figure 3 as "n=30" for each treatment concentration. No replication was reported.	
Domain 5: Outcome Ass	Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.				
Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 4728405 Table: 1 of 4

		contin	nued from p	revious page				
Study Citation:	Pradhan, A., of Caenorha	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	legans; Wild type Bristol-N2; Larvae				
Health Outcome:	Nutritional &	& Metabolic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	4728405	-						
Domain		Metric Rating Comments						
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.				
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.				
Domain 6: Confounding	y / Variable Co	ntrol						
Domain of Comountaing	Metric 19:	Confounding Variables in Test	High	No reported differences that would indicate confounding variables				
		Design and Procedures	i iigii	To reported universities that would indicate confounding variables.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.				
	Metric 22:	Reporting of Data	High	The presentation of 'relative nile red intensity' for fat staining is presented in figure 3." Lipid quantification was done by measuring the fluorescence intensity of the individual worms and normalizing the values to their respective body area".				
	Metric 23:	Explanation of Unexpected Outcomes	Low	Scatter plots are used to represent the fat staining among treatment concentrations, so traditional error terms are not available.				
Additional Comments:	Results of lip	pid staining assay (Fig 3) showed that DEH	IP significant	ly increased lipid content following 1 mM exposure.				
Overall Qualit	ty Detern	nination	High					

Diethylhexyl Phthalate

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HERO ID: 4728405 Table: 2 of 4

Study Citation:	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan						
Duration: Exposure Route, Media. Path:	of Caenorha Overall Dura Terrestrial; (Dverall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Ferrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Wild type Bristol-N2; Larvae					
Health Outcome:	Developmen	nt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	4728405						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.			
	Metric 2:	Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.			
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) listed at 99.5%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.			
	Metric 5:	Negative Control Response	High	Figure 3 (page 5/8) lists the control responses for body area effects.			
	Metric 6:	Randomized Allocation	Low	No random allocation was described.			
Domain 3: Exposure Ch	aracterization		TT' 1				
	Metric /:	Experimental System/Test Media	High	Test media preparation was described adequately in section 2.1.			
	Metric 8:	Consistency of Exposure	High	The two treatment concentrations appear to be administered similarly.			
	Metric 9:	Administration Measurement of Test Substance	Low	No compounds were measured for analytical verification of treatment concentrations,			
	Matria 10.	Concentration	II: -1-	concentrations are reported as nominal.			
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr duration for the assessment of body area appears to be appropriate for C. ele- gans.			
	Metric 11:	Number of Exposure Groups/	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50			
		Spacing of Exposure Levels		value for this species.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for C. elegans is a solid.			
Domain 4: Test Organis	m						
en e	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration were not reported in the methods but are available in figure 3 as "n=30" for each treatment concentration. No replication was reported.			
Domain 5: Outcome As	Motria 16	A dequeey of Test Conditions	Madin	Environmental registring the test (terms light could) some actions at the state of the			
	Metric 16:	Adequacy of rest Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.			
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.			
		Cont	inued on ney	xt page			

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Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 4728405 Table: 2 of 4

		contin	nued from p	previous page			
Study Citation:	Pradhan, A., of Caenorha	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	ly authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Caer	norhabditis e	legans; Wild type Bristol-N2; Larvae			
Health Outcome:	Developmen	nt/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	4728405	-					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	No reported differences that would indicate confounding variables.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.			
Domain 7: Data Presen	tation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.			
	Metric 22:	Reporting of Data	High	Data and results describing the body area assessment are shown in Figure 3C and in the text on page 379.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Scatter plots are used to represent the body area among treatment concentrations, so traditional error terms are not available.			
Additional Comments: Overall Ouali	The body are	ea measurements indicated that there was n	o significant High	difference in C. elegans development upon exposure to DEHP (Fig. 3C).			

Duration: O - 4 days (U-Son): Exposure Duration: O - 4 days (U-Son): Exposure Duration: O - 4 days (U-Son): Exposure Active Terreterized of interest in exposure water, but mable to determine exact uptake route) Media, Furt: Track Species Age: Invertebrate: Worms (e.g., Annelids, Nematodes): Caenorhabditis elegans: Wild type Bristol-N2: Larvae Ineuto Duration: Domain 4: Test Substance Identity Metric 2: Test Substance Durity Metric 2: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Design Metric 4: Negative Control Response Low The exocution adjoc duration of the adjoc	Study Citation:	Pradhan, A., of Caenorhal	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.					
Trans, Species, Age: Invertebrate: Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Wild type Bristol-N2; Larvae Health Outcome: Directly phthalate (DEHP) Health Outcome Directly phthalate (DEHP) HERO Dir. 4728405 Domain 1: Test Substance Metric 1: Test Substance Identity High Metric 2: Authors have the CAS number: molecular weight, and structure of the compound listed. Metric 3: Domain 1: Test Substance Metric 2: Test Substance Controls Uninformative Americ A concurrent negative control group was not included or reported. Metric 5: Negative Controls Domain 2: Test Design Metric 6: Randomized Allocation Low No nation abactine of the survival bioassay (first para- graph of section 3). Domain 3: Exposure Characterization Low No random abactine was described. Domain 3: Exposure Characterization Metric 6: Randomized Media High Test media preparation was described. Domain 4: Test Organism Metric 1: Stapseure Daration and Frequency High The four treatment concentration on advecting anominal. Metric 6: Randomized Motion No No compound were measured for analytical verification of treatment concentrations and eccentanadvered anominal.	Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; C	Cell Culture Media; Not determined by study	: 0 - 4 days (0-96h) authors (i.e., chemical of	f interest in exposure water, but unable to determine exact uptake route)			
Chemical: HEKO ID: Dis-ethylikexyl puthalate (DEHP) HEKO ID: 4728405 Domain Metric Rating Comments Domain 1: Test Substance Test Substance Identity High Authors have the CAS number, molecular weight, and structure of the compound listed. The source was listed, but the performing lab did not analytically verify the test sub- stance. Domain 2: Test Design Keatric 2: Test Substance Purity High Purity from source (signa) was listed at 99.5%. Domain 3: Test Design Keatric 5: Negative Controls Uninformative Low A concurrent negative control group was not included or reported. Metric 6: Randomized Allocation Low The results did not state the results of the control for the survival bioassay (first para- graph of section 3). Domain 3: Exposure Characterization Keptrinental System/Test Media High Test media preparation was described adequately in section 2.1. Preparation No random allocation was described adequately in section 2.1. Preparation Metric 7: Experimental System/Test Media Preparation High Test media preparation was described adequately in section 2.1. Preparation Metric 10: Exposure Duration and Frequency High The four treatment concentrations are preported as nominal. Metric 11: Number of Exposure Coupl	Taxa, Species, Age: Health Outcome:	Invertebrate; Mortality	nvertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae Aortality					
Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Source Test Substance Identity Metric 2: Test Substance Source High Low Authors have the CAS number, molecular weight, and structure of the compound listed. Metric 3: Test Substance Purify High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Design Metric 4: Negative Controls Uninformative Low A concurrent negative control group was not included or reported. Metric 5: Negative Controls Uninformative Low A concurrent negative control group was not included or reported. Domain 3: Exposure Characterization Metric 6: Randomized Allocation Low No random allocation was described. Domain 3: Exposure Characterization Metric 9: Metric 7: Experimental System/Test Media Preparation Metric 9: High Metric 9: The four treatment concentrations appeared to be appropriate for C. elegans. and concentrations were reported as found was described adequately in section 2.1. Metric 10: Exposure Duration and Frequency Metric 11: High Metric 9: The source was reported as maintain. Metric 10: Exposure Duration and Frequency Metric 11: High Metric 12: Test onegative concentrations were reported as molinal. Metric 12: Test organism Metric 12: Test organism Characteristics Met	Chemical: HERO ID:	Di-ethylhexy 4728405	vl phthalate (DEHP)					
Domain 1: Test Substance Metric 1: Test Substance Identity High Authors have the CAS number, molecular weight, and structure of the compound listed. Metric 2: Test Substance Source Low The source was listed, but the performing lab did not analytically verify the test sub-stance. Metric 3: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Design Metric 6: Negative Controls Uninformative A concurrent negative control group was not included or reported. Metric 5: Negative Control Response Low The results did not state the results of the control for the survival bioassay (first paragraph of section 3). Metric 6: Randomized Allocation Low No random allocation was described. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Metric 9: Consistency of Exposure High The four treatment concentrations supported to be administered similarly. Administration Low No compounds were neasured for analytical verification of treatment concentrations, and concentrations were reported as nominal. Metric 11: Number of Exposure Groups/ High The concentrations were reported as nominal. Metric 12: Testing at or Below Solubility Limi	Domain		Metric	Rating	Comments			
Metric 1: Test Substance Identity High Metric 2: Authors have the CAS number, molecular weight, and structure of the compound listed. Netric 3: Metric 2: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Substance Purity A concurrent negative control group was not included or reported. The results did not state the results of the control for the survival bioassay (first para- graph of section 3). Metric 6: Randomized Allocation Low No random allocation was described. Domain 3: Experimental System/Test Media High Test media preparation was described adequately in section 2.1. Preparation Metric 7: Experimental System/Test Media High The four treatment concentrations appeared to be administered similarly. Administration Metric 8: Consistency of Exposure High The four treatment concentrations appeared to be administered similarly. Administration Metric 10: Experimental System/Test Media High The four treatment concentrations appeared to be administered similarly. Administration Metric 19: Metric 19: Experimental System/Test Media High Metric 10: Exposure Groups/	Domain 1: Test Substand	ce						
Metric 3: Test Substance Purity High Purity from source (sigma) was listed at 99.5%. Domain 2: Test Design Metric 4: Negative Controls Uninformative A concurrent negative control group was not included or reported. Metric 5: Negative Control Response Low The results did not state the results of the control for the survival bioassay (first paragraph of section 3). Metric 6: Randomized Allocation Low No random allocation was described. Domain 3: Exposure Characterization Preparation Preparation Metric 8: Consistency of Exposure High Test media preparation was described adequately in section 2.1. Preparation Preparation Metric 9: Metric 10: Exposure Outration and Prequency Metric 10: Exposure Duration and Prequency High The four treatment concentrations, and concentrations were selected at anominal. Metric 11: Number of Exposure Duration A 48-th duration for this acute bioasay appeared to be appropriate for C. elegans. Metric 12: Test Organism Metric 13: Test Organism Characteristics High Domain 4: Test Organism Characteristics High The s		Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	Authors have the CAS number, molecular weight, and structure of the compound listed. The source was listed, but the performing lab did not analytically verify the test sub-			
Domain 2: Test Design Metric 4: Negative Controls Uninformative A concurrent negative control group was not included or reported. Metric 5: Negative Control Response Low The results did not state the results of the control for the survival bioassay (first paragraph of section 3). Metric 6: Randomized Allocation Low No random allocation was described. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Test media preparation as described adequately in section 2.1. Metric 8: Consistency of Exposure High The four treatment concentrations appeared to be administered similarly. Administration Administration Low No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal. Metric 10: Exposure Duration and Frequency High The concentrations were selected above and below a previously published LC50 value for this socies. Metric 11: Number of Exposure Levels N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High The source was reported as from the Karolinski Institute. The animal care was well described. Metric 14: Acclimatization and Pretreatment High		Metric 3:	Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.			
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Metric 6:Randomized AllocationLowNo random allocation was described.Domain 3: Exposure CharacterizationMetric 7:Experimental System/Test MediaHighTest media preparation was described adequately in section 2.1. PreparationMetric 7:Experimental System/Test MediaHighThe four treatment concentrations appeared to be administered similarly. AdministrationMetric 8:Consistency of ExposureHighThe four treatment concentrations appeared to be administered similarly. AdministrationMetric 9:Metasurement of Test SubstanceLowNo compounds were measured for analytical verification of treatment concentrations, and concentrations were seported as nominal. Metric 10:Exposure Duration and FrequencyMetric 10:Exposure Duration and FrequencyHighA 48-th duration for this acute bioassay appeared to be appropriate for C. elegans. Metric 11:Number of Exposure Groups/ Spacing of Exposure CurvelsHighThe concentrations were selected above and below a previously published LC50 value for this species. Metric 12:Domain 4: Test OrganismMetric 13:Test Organism CharacteristicsHighMetric 14:Acclimatization and Pretreatment ConditionsHighSection 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (inius the compound).Metric 15:Number of Organisms and Replicates per GroupMediumTwenty stage L1 worms were used per replicate and there were five replicates per treat- met concentration.Domain 5: Outcome Assessment Metric 16:Adequacy of Test ConditionsMediumEnviron		Metric 5:	Negative Control Response	Low	The results did not state the results of the control for the survival bioassay (first para- graph of section 3).			
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Test media preparation was described adequately in section 2.1. Preparation Metric 8: Consistency of Exposure High The four treatment concentrations appeared to be administered similarly. Administration Metric 9: Measurement of Test Substance Low No compounds were measured for analytical verification of treatment concentrations, and concentration of the substance Metric 10: Exposure Duration and Frequency High The concentrations were reported as nominal. Oncentration for this acute bioassay appeared to be appropriate for C. elegans. Metric 11: Number of Exposure Duration and Frequency High The concentrations were selected above and below a previously published LC50 value for this species. Metric 12: Testing at or Below Solubility Limit N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High The source was reported as from the Karolinski Institute. The animal care was well described. Metric 15: Number of Organism and Retric 15: Medium Theway EL Worms were used per replicate and there were five replicates per treat- ment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental		Metric 6:	Randomized Allocation	Low	No random allocation was described.			
Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media High Test media preparation was described adequately in section 2.1. Metric 7: Experimental System/Test Media High The four treatment concentrations appeared to be administered similarly. Administration Metric 9: Measurement of Test Substance Low No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal. Metric 10: Exposure Duration and Frequency High The concentrations were selected above and below a previously published LC50 value for this species. Metric 11: Number of Exposure Groups/ High The source was reported as from the Karolinski Institute. The animal care was well described. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organism and Medium Twenswere used per replicate and there were five replicates per treatment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should <td></td> <td>, . ..</td> <td></td> <td></td> <td></td>		, . . .						
Metric 7: Experimental System/Test Media Fright Test media preparation was described adequately in section 2.1. Preparation Preparation Preparation The four treatment concentrations appeared to be administered similarly. Administration Metric 8: Consistency of Exposure High The four treatment concentrations appeared to be administered similarly. Metric 9: Measurement of Test Substance Low No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal. Metric 10: Exposure Duration and Frequency High A 48-hr duration for this acute bioassay appeared to be appropriate for C. elegans. Metric 11: Number of Exposure Levels for this species. Metric 12: Testing at or Below Solubility Limit N/A Domain 4: Test Organism Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High Conditions Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration. Domain 5: Outcome Assessment Medium Twenty stage L1 worms were used per replicate and there were five reported, but should	Domain 3: Exposure Cha	aracterization	Environmental Scottant (Teat Madia	II: -h				
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Administration Administration Metric 9: Measurement of Test Substance Low No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal. Metric 10: Exposure Duration and Frequency High A 48-hr duration for this acute bioassay appeared to be appropriate for C. elegans. Metric 11: Number of Exposure Groups/ High The concentrations were selected above and below a previously published LC50 value for this species. Metric 12: Testing at or Below Solubility Limit N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organisms and Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 8:	Consistency of Exposure	High	The four treatment concentrations appeared to be administered similarly.			
Metric 10: Exposure Duration and Frequency High A 48-hr duration for this acute bioassay appeared to be appropriate for C. elegans. Metric 11: Number of Exposure Groups/ High The concentrations were selected above and below a previously published LC50 value for this species. Metric 12: Testing at or Below Solubility Limit N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High The source was reported as from the Karolinski Institute. The animal care was well described. Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organisms and Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment ment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 9:	Administration Measurement of Test Substance	Low	No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal			
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels High The on concentrations were selected above and below a previously published LC50 value for this species. Metric 12: Testing at or Below Solubility Limit N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organisms and Replicates per Group Medium Twenty stage L1 worms were used per replicate and there were five replicates per treat- ment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 10:	Exposure Duration and Frequency	High	A 48-hr duration for this acute bioassay appeared to be appropriate for C elegans			
Metric 12: Testing at or Below Solubility Limit N/A The growth media for C. elegans is a solid. Domain 4: Test Organism Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High Conditions Metric 15: Number of Organisms and Metric 15: Number of Organisms and Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration. The source may are used being the test (temp, light cycle) were not reported, but should		Metric 11:	Number of Exposure Groups/	High	The concentrations were selected above and below a previously published LC50 value for this species			
Domain 4: Test Organism Metric 13: Test Organism Characteristics High The source was reported as from the Karolinski Institute. The animal care was well described. Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organisms and Replicates per Group Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for C. elegans is a solid.			
Metric 13: Test Organism Characteristics High The source was reported as from the Karolinski Institute. The animal care was well described. Metric 14: Acclimatization and Pretreatment High Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound). Metric 15: Number of Organisms and Replicates per Group Medium Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration. Domain 5: Outcome Assessment Medium Environmental variables during the test (temp, light cycle) were not reported, but should	Domain 4: Test Organis	m						
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Metric 15: Number of Organisms and Replicates per Group Medium Twenty stage L1 worms were used per replicate and there were five replicates per treat- ment concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).			
Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should		Metric 15:	Number of Organisms and Replicates per Group	Medium	Twenty stage L1 worms were used per replicate and there were five replicates per treat- ment concentration.			
Metric 16: Adequacy of Test Conditions Medium Environmental variables during the test (temp, light cycle) were not reported, but should	Domain 5: Outcome Ass	resement						
not impact results.	Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.			
Continued on next page			С	ontinued on next page .				

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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 4728405 Table: 3 of 4

		CO	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Pradhan, A., of Caenorha Overall Dura Terrestrial; C Invertebrate; Mortality Di-ethylhexy	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae Mortality Di-ethylhexyl phthalate (DEHP)				
Domain						
	Metric 17:	Outcome Assessment Methodology	Medium	"the survival assay which did not show any lethality at 0.1, 1, 10 and 100 mM of DEHP and DEP over 48 h time period (data not shown)."		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.		
Domain 6: Confoundir	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences that would indicate confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.		
Domain 7: Data Preser	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Negative findings occurred across all groups. "the survival assay which did not show any lethality at 0.1, 1, 10 and 100 mM of DEHP and DEP over 48 h time period (data not shown)."		
	Metric 22:	Reporting of Data	Uninformative	The authors presented no data on the mortality study.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not report any data for these mortality assays and did not expect the treatment concentrations to result in no mortality.		

Overall Quality Determination

Uninformative

Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media. Path:	Pradhan, A., of Caenorha Overall Dura Terrestrial; C	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic Di-ethylhexy 4728405	Worms (e.g., Annelids, Nematodes); <i>Caer</i> Biomarkers (exposure and effect)-Cell sig <i>i</i> l phthalate (DEHP)	<i>iorhabditis e</i> naling/functi	<i>legans</i> ; Wild type Bristol-N2; Larvae on-Oxidative stress (including redox biology)		
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.		
	Metric 2:	Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.		
	Metric 3:	Test Substance Purity	Hıgh	Purity from source (sigma) listed at 99.5%.		
Domain 2: Test Design						
_ 5.1.0.1 2. 1000 Doolgii	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.		
	Metric 5:	Negative Control Response	High	Figure 1 (page 4/8) lists the control responses for target genes. Control gene expression was considered 1, with treatment concentrations demonstrating relative expression normalized by the control response.		
	Metric 6:	Randomized Allocation	Low	No random allocation was described.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.		
	Metric 8:	Consistency of Exposure Administration	High	The two treatment concentrations appear to be administered similarly.		
	Metric 9:	Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, concentrations are reported as nominal.		
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr duration for this gene expression bioassay appear to be appropriate for C. ele- gans.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50 value for this species.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for C. elegans is a solid.		
Domain 4: Test Organis	m Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well		
	Metric 14:	Acclimatization and Pretreatment	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound)		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration and replicates were not reported in the meth- ods but in figure 1 caption, it states "n=6" for each treatment concentration.		
Domain 5: Outcome As	recement					
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.		
Continued on next page						

PUBLIC RELEASE DRAFT

May 2025

Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 4728405 Table: 4 of 4

		conti	nued from p	previous page				
Study Citation:	Pradhan, A., of Caenorha	radhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan f Caenorhabditis elegans. Chemosphere 190:375-382.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	ly authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caer	10rhabditis e	legans; Wild type Bristol-N2; Larvae				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sig	naling/functi	on-Oxidative stress (including redox biology)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	4728405							
Domain	Metric Rating Comments							
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.				
	Metric 18:	Consistency of Outcome	High	The outcome assessment appeared to be assessed consistently among treatment groups.				
		Assessment						
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	No reported differences that would indicate confounding variables.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.				
	Metric 22:	Reporting of Data	High	Relative expression is reported in figure 2 (page 4/8) for the target genes.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Standard Error of the Mean are reported for each treatment concentration and gene.				
Additional Comments:	Expression of with lipid model.	of lipid metabolism and stress response gen etabolism, including fasn-1, pod-2, fat-5, a st-4 were upregulated, while ctl-1, cdf-2 ar	es were asses cs-6 and sbp nd the heat sh	ssed by exposing worms at L1 stage to 1 and 10 mM DEHP for 48 h."Genes associated -1, and vitellogenin were upregulated. Among the stress response genes, ced-1 wah-1, nock proteins (hsp-16.1, hsp-16.48 and sip-1) were downregulated".				
Overall Qualit	ty Determ	nination	High					

Study Citation:	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression					
Duration:	in the soil ne Overall Dura	ematode Caenorhabditis elegans. Toxicolo ation: 0 - 4 days (0-96h); Exposure Durati	bgy 237(1-3):126-133. ion: 0 - 4 days (0-96h)			
Exposure Route, Modia Dath:	Terrestrial; C	Cell Culture Media; Not determined by stu	idy authors (i.e., chemical of ir	iterest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate:	Worms (e.g. Annelids Nematodes): Cae	enorhabditis elegans [.] Wild Tyr	e Bristol Strain [.] Adult		
Health Outcome:	Developmen	Development/Growth				
Chemical:	Di-ethylhexy	/l phthalate (DEHP)				
HERO ID:	698288	-				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice		TT ' 1			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.		
	Metric 2:	Test Substance Source	High	The source was identified as Sigma-Aldrich.		
	Metric 3:	Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment.		
Domain 2: Test Design						
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	naracterization					
	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
		Preparation		test concentrations.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups were adequate for a dose response.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via soil.		
Domain 4: Test Organis	m					
Domain 1. 10st Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Group		ize toxicological effects.		
Domain 5: Outcom- A-	unanemant.					
Domain 5: Outcome As	Metric 16	A deguacy of Test Conditions	Цiah	Environmental conditions of the test system wars conducing to maintenance of according		
	wieute to:	Aucquacy of rest Conditions	rigi	health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
Continued on next page						

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Diethylhexyl Phthalate

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		COI	ntinued from previous _l	page	
Study Citation:	Roh, J., Jung	, I., Lee, J., Choi, J. (2007). Toxic effects of	di(2-ethylhexyl)phthalat	te on mortality, growth, reproduction and stress-related gene expression	
	in the soil ne	matode Caenorhabditis elegans. Toxicology 2	237(1-3):126-133.		
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-96h)		
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:					
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caenor	habditis elegans; Wild T	ype Bristol Strain; Adult	
Health Outcome:	Development	t/Growth			
Chemical:	Di-ethylhexy	l phthalate (DEHP)			
HERO ID:	698288	•			
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.	
		Assessment			
Domain 6: Confounding	y / Variable Cor	atrol			
Domain of Comounding	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups	
	metric 19.	Design and Procedures	Ingn	There were no reported anterences among the study groups.	
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Present	ation and Analy	ys1s			
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Commentar	Landfill soils	wara also testad			
Aduitional Comments.	Lanum sons were also tested.				

Overall Quality Determination

Uninformative

Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult Mechanistic-Cell signaling/function Di-ethylbexyl phthalate (DEHP)				
HERO ID:	698288				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name	
	Metric 2:	Test Substance Source	High	Source identified as Sigma-Aldrich.	
	Metric 3:	Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment	
Domain 2: Test Design					
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 2: Exposure Ch	aractorization				
Domain 5: Exposure Ch	Matria 7	Evenimental System/Test Madia	Low		
	Metric 7:	Branaration	LOW	test concentrations	
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups.	
	Matria O.	Administration			
	Metric 9:	Concentration	Low	Exposure concentrations were not measured	
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups were adequate for a dose response	
		Spacing of Exposure Levels			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organist	n				
Domain 1. 10st Organisi	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported	
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms	
		Conditions			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-	
		Replicates per Group		ize toxicological effects	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest	
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups	
		Assessment			

Diethylhexyl Phthalate

		COI	ntinued from previous	page			
Study Citation:	Roh, J., Jung	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression					
	in the soil ne	in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study a	authors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caenor	<i>habditis elegans</i> ; Wild T	ype Bristol Strain; Adult			
Health Outcome:	Mechanistic-	-Cell signaling/function					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	698288	698288					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.			
Domain 7. Data Dragant	ation and Anal						
Domain 7. Data Fresent	Motrio 21	ysis Statistical Matheda	Uninformativa	Statistical analysis was not conducted			
	Metric 21:	Statistical Methods		Statistical analysis was not conducted			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes,					
Additional Comments:	Landfill soils	s also tested					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 698288 Table: 3 of 4

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 698288					
Chemical: HERO ID:						
Domain		Metric	Rating	Comments		
Domain 1: Test Substar	nce					
	Metric 1:	Test Substance Identity	High	Chemical was identified by name		
	Metric 2:	Test Substance Source	High	Source identified as Sigma-Aldrich.		
	Metric 3:	Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment		
Domain 2: Test Design						
U	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3. Exposure C	horacterization					
Domain 5. Exposure C	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
		Preparation	2011	test concentrations		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups.		
	Metric 9	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	metrie 9.	Concentration	Low	Exposure concentrations were not incustrice		
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups were adequate for a dose response		
	14 - 10	Spacing of Exposure Levels	27/4			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.		
Domain 4: Test Organis	sm					
8	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms		
	Metric 15	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to observe and		
	Metho 15.	Replicates per Group	Wedium	ize toxicological effects		
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest		
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups		

Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression						
	in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133.						
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Wild Type Bristol Strain; Adult						
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	698288						
Domain	Metric		Rating	Comments			
Domain 6: Confounding / Variable Control							
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Presentation and Analysis							
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes,			
Additional Comments:	Landfill soils also tested						

Overall Quality Determination

Uninformative
Study Citation: Duration: Exposure Route, Media, Path:	Citation: Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133. on: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) ure Route, Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact up . Path: Path:							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 698288	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult Mortality Di-ethylhexyl phthalate (DEHP) 608288						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Chemical was identified by name				
	Metric 2:	Test Substance Source	High	Source identified as Sigma-Aldrich.				
	Metric 3:	Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations				
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured				
	Metric 10 [.]	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups were adequate for a dose response				
		Spacing of Exposure Levels	8					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.				
Domain 4: Test Organis	m							
0	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported				
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest				
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups				

Diethylhexyl Phthalate

		contin	ued from previo	us page			
Study Citation:	Roh, J., Jung in the soil ne	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression n the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caend	orhabditis elegans	; Wild Type Bristol Strain; Adult			
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	698288	698288					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Con	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes,			
Additional Comments:	Landfill soils	s also tested					
Overall Qualit	ty Detern	nination	Medium				

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Study Citation:	Shin, N., Cu	Shin, N., Cuenca, L., Karthikraj, R., Kannan, K., Colaiácovo, M. P. (2019). Assessing effects of germline exposure to environmental toxicants by high-						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h): Exposure Duration	1: 0 - 4 days (0-9	(6h)				
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	y authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	,							
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Larvae							
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Cell sign	aling/function-G	enotox (including DNA repair)				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	5043459							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and structure.				
	Metric 2:	Test Substance Source	High	The test substance identity was verified by GC-MS.				
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups. Multiple strains were tested, but it is unclear which strain results were reported for various tests.				
	, . ,.							
Domain 3: Exposure Ch	Matria 7	Even animantal System / Test Madia	Low					
	Metric 7:	Preparation	LOW	the study provided only limited details on the measures taken to appropriately prepare				
	Metric 8.	Consistency of Exposure	Low	Only general methods of exposure administration were reported so assessment was				
	incure of	Administration	Low	difficult to determine.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
		Concentration						
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/	High	Four concentrations over an adequate range were used.				
	16 . 1 . 10	Spacing of Exposure Levels						
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organis	m							
0	Metric 13:	Test Organism Characteristics	Low	The original source was not reported.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions appeared to be the same for control and exposed organisms, but few details were provided.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Three replicate exposures were reported.				
		* ··· F · · · · F						

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 5043459 Table: 1 of 1

continued from previous page							
Study Citation:	Shin, N., Cu	Shin, N., Cuenca, L., Karthikraj, R., Kannan, K., Colaiácovo, M. P. (2019). Assessing effects of germline exposure to environmental toxicants by high-					
	throughput s	throughput screening in C. elegans. Pl o S Genetics 15(2):e1007975.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	y authors (i.e., cl	nemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis elegai	ns; Larvae			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	aling/function-C	Genotox (including DNA repair)			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5043459						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group			
	in most cases. It was unclear if there were multiple tests or if only the results from one concentration were reported for some assessed endpoints.						
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	Multiple stra	ins were tested, but it is unclear which strai	n results were re	ported. This form was for the DEHP mechanistic outcome reported in Fig. 1A.			

Overall Quality Determination

Medium

Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration: Exposure Route, Media Path:	Overall Dura Terrestrial; C	ntion: 0 - 4 days (0-96h); Exposure Duratio Cell Culture Media; Not determined by stud	n: 0 - 4 days ly authors (i.	s (0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae					
Health Outcome:	Mechanistic-Oxidative stress (including redox biology) Di athulaarul abthalata (DEHD)					
HERO ID:	2215375					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce		TT' 1			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
1	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was reported and adequate for the study type, but there was no "No Effect level"		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and spacing of exposure levels were adequate for a dose response but there was no "No Effect level"		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium		
Domain 4: Test Organisi	m					
U	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms		
	Metric 15:	Conditions Number of Organisms and	Low	The number of test organisms was not reported, repeated tests were used as replicates		
		Replicates per Group		(n=3)		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest		
		Conti	nued on ne	xt page		

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Diethylhexyl Phthalate

	continued from previous page						
Study Citation:	Tseng, I. L., AFD neuron	Iseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; wild type; Larvae			
Health Outcome:	Mechanistic	-Oxidative stress (including redox biology)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2215375						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.			
Domain 7. Data Present	ation and Anal	train.					
Domain 7: Data Present	Motrio 21:	Statistical Matheda	Uich	Statistical matheda wara adaguataly, dagarihad			
	Metrie 22:	Benerting of Data	High	Statistical methods were adequately described			
	Metric 22:	Reporting of Data	пigii	and were adequate to determine values for the endpoint(s) of interest			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	s: Ascorbic acid pretreatment, reactive oxygen species						
Overall Quality Determination High							

Study Citation: Duration: Exposure Route,	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Di-ethylhexy 2215375	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae Mechanistic-Oxidative stress (including redox biology) Di-ethylhexyl phthalate (DEHP) 2215375					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2. Test Design							
Domain 2: Test Design	Metric 4	Negative Controls	High	Study authors reported using a concurrent negative control			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups			
	incure o.	Tundonii2eu Tinoeuton	Lon	Researchers du not report now organisms were anotated to study groups.			
Domain 3: Exposure Ch	aracterization						
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was reported and adequate for the study type, but there was no "No Effect level"			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and spacing of exposure levels were adequate for a dose response but there was no "No Effect level"			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium			
Domain 4: Test Organis	Matria 12.	Test Organism Characteristics	U:~h	The test superious man adapted is described and more abtained from a well-bla			
	Metric 14	A colimatization and Protreatment	пign Цiab	The test organisms were adequately described and were obtained from a reliable source.			
	Metho 14.	Conditions	nıgii	an pretreatment conditions were the same for control and exposed organisms			
	Metric 15:	Number of Organisms and	Low	The number of test organisms was not reported, repeated tests were used as replicates			
		Replicates per Group		(n=3)			
Domain 5. Outagers A.	agament						
Domain 5: Outcome Ass	Metric 16.	A dequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism			
	Matria 17	Outcome Assessment Methodalary	Tingii U: ~h	health			
	Matri - 19	Consistency of Outcome	High	i ne outcome assessment methodology reported the intended outcome of interest			
	Metric 18:	Assessment	High	outcomes were assessed consistently across study groups			
		Conti	inued on ne	xt nage			

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Tseng, I. L.,	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and				
	AFD neuron	s through oxidative stress in Caenorhabditis	s elegans. P	LoS ONE 8(12):e82657.		
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	s (0-96h)		
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	y authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; wild type; Larvae		
Health Outcome:	Mechanistic	-Oxidative stress (including redox biology)				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures	-			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	veie				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Matria 22:	Benerting of Data	High	Data for averaging related for dings were presented for each treatment and control group		
	Metric 22:	Reporting of Data	пign	and were adequate to determine values for the endpoint(s) of interest		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	Ethanol pret	Ethanol pretreatment, reactive oxygen species				

Overall Quality Determination

High

Study Citation:	Tseng, I. L., AFD neuron	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration: Exposure Route, Madia Dath:	Overall Dura Terrestrial; C	tion: 0 - 4 days (0-96h); Exposure Duratio Cell Culture Media; Not determined by stu	on: 0 - 4 days dy authors (i.	(0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age: Health Outcome:	Invertebrate; Mechanistic-	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)					
Chemical: HERO ID:	Di-ethylhexy 2215375	l phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	aractarization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type			
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration tested			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium			
Domain 4: Test Organis	m						
6	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups			

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Study Citation:	Tseng, I. L., AFD neuron	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	y authors (i	e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate	Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; wild type; Larvae			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Genotox	(including	DNA repair)			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2215375	2215375					
Domain		Metric Rating Comments					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	gene express	sion, fig.4					
Overall Quali	ty Deterr	nination	High				

Study Citation:	Tseng, I. L., AFD neuron	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration: Exposure Route, Modia, Pathy	Overall Dura Terrestrial; C	ation: 0 - 4 days (0-96h); Exposure Duratic Cell Culture Media; Not determined by stu	on: 0 - 4 days dy authors (i.	(0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age: Health Outcome:	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae Behavioral					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	Chemical was identified by name		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups		

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Study Citation:	Tseng, I. L.,	Cseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and				
	AFD neuron	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration:	Overall Dur	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	s (0-96h)		
Exposure Route,	Terrestrial; (Cell Culture Media; Not determined by stud	y authors (1.	e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	<i>legans</i> ; wild type; Larvae		
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric Rating Comments				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	locomotor a	nd thermotaxis				
Overall Quality Determination High						

Study Citation: Duration: Exposure Route, Media Path:	Tseng, I. L., AFD neuron Overall Dura Terrestrial; C	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C. s through oxidative stress in Caenorhabditi ation: 0 - 4 days (0-96h); Exposure Duratio Cell Culture Media; Not determined by stud	, V.H. (2013 s elegans. Pl n: 0 - 4 days ly authors (i.). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and LoS ONE 8(12):e82657. s (0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	norhabditis e	elegans; DA 1267; Larvae
Health Outcome:	Mechanistic	Neurotoxicology-Ocular and Sensory		
Chemical:	Di-ethylhexy	vl phthalate (DEHP)		
HERO ID:	2215375			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was reported and adequate for the study type, but there was no "No Effect level."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and spacing of exposure levels were adequate for a dose response but there was no "No Effect level."
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.
Domain 4: Test Organis	m			
O	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	M + 1 15	Conditions	T	
	Metric 15:	Number of Organisms and	Low	The number of test organisms was not reported, and repeated tests were used as repli- cates $(n-3)$
		Replicates per Group		cates (n=5).
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment		
		Conti	nued on nex	xt page

Diethylhexyl Phthalate

		contin	ued from p	previous page		
Study Citation:	Tseng, I. L.,	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and				
	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	y authors (i.	.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caena	orhabditis e	elegans; DA 1267; Larvae		
Health Outcome:	Mechanistic	Neurotoxicology-Ocular and Sensory				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric Rating Comments				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7. Data Present	ation and Anal	veic				
Domain 7. Data Hesen	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Penorting of Data	High	Date for exposure related findings were presented for each treatment and control group		
	Wieute 22.	Reporting of Data	nıgii	and were adequate to determine values for the endpoint(s) of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluati	on form is for assessment of AFD thermose	ensory neur	ons.		

Overall Quality Determination

High

Study Citation:	Tseng, I. L., AFD neuron	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C s through oxidative stress in Caenorhabdit	., V.H. (2013) is elegans. PI). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and LoS ONE 8(12):e82657.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	(0-96h)					
Exposure Route,	Terrestrial; C	Invertebrate: Worms (e.g. Appelids, Nematodes): <i>Caeporhabditis alegans</i> ; wild type: Larvae							
Media, Path: Taxa Spacios Ago:	Invartabrata								
Health Outcome:	Behavioral	worms (e.g., Annends, Nennatodes), Cael	nornaballis e	leguns, which type, Lat vae					
Chemical:	Di-ethylhexy	l phthalate (DEHP)							
HERO ID:	2215375	- p							
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	High	Chemical was identified by name					
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.					
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.					
Domain 2: Test Design									
c c	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control					
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate					
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.					
Domain 2. Expedium Ch	anastanization								
Domain 5: Exposure Ch	Matria 7	Even montal System/Test Madia	Iliah						
	Metric 7:	Preparation	Fign	adequate detail					
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups					
		Administration	ingii	enpositios noto administered consistenti y actoss stady groups					
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured					
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type					
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration tested					
		Spacing of Exposure Levels							
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium					
Domain 4: Test Organis	m								
5	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.					
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms					
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)					
Domain 5: Outcome As	sessment								
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest					
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups					

		contin	ued from p	orevious page		
Study Citation:	Tseng, I. L.,	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C.,	V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and		
	AFD neuron	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; (Cell Culture Media; Not determined by stud	y authors (i.	.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; wild type; Larvae		
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric Rating Comments				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	lycic				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Peporting of Data	High	Date for exposure related findings were presented for each treatment and control group		
	Metric 22.	Reporting of Data	nıgıı	and were adequate to determine values for the endpoint of interest		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	ethanol pret	reatment, locomotor and thermotactic, fig.6				
	- manor prou	and method and the method where the second sec				
Overall Qualif	v Deterr	nination	High			
Vician Quan	y Duun	1111411011	111gii			

Study Citation:	Tseng, I. L.,	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C s through ovidative stress in Caenorhabdit	., V.H. (2013)). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and			
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; C	ation: 0 - 4 days (0-96h); Exposure Duratio Cell Culture Media; Not determined by stu	on: 0 - 4 days dy authors (i.	(0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Cae	norhabditis e	legans; DA 1267; Larvae			
Health Outcome:	Mechanistic-	Neurotoxicology-Ocular and Sensory					
Chemical:	Di-ethylhexy	i-ethylhexyl phthalate (DEHP) 215375					
HERO ID:	2215375			2			
Domain Domain 1: Test Substan		Metric	Rating	Comments			
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	The chemical was identified by name			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.			
Domain 2: Test Design		•		· · · · · ·			
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using a concurrent negative control			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type.			
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration was tested.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, and repeated tests were used as replicates (n=3).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.			

Diethylhexyl Phthalate

		contin	ued from p	orevious page		
Study Citation:	Tseng, I. L.,	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C.,	V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and		
	AFD neuron	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	y authors (i.	.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate	Worms (e.g., Annelids, Nematodes); Caena	orhabditis e	elegans; DA 1267; Larvae		
Health Outcome:	Mechanistic	-Neurotoxicology-Ocular and Sensory				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric Rating Comments				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion form is for ethanol pretreatment and AF	D thermose	ensory neurons (fig.7).		
Overall Quali	ty Deterr	nination	High			

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Study Citation:	Tseng, I. L., AFD neuron	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C s through oxidative stress in Caenorhabdit	., V.H. (2013) is elegans. PI). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and LoS ONE 8(12):e82657.
Exposure Route.	Terrestrial C	inon. 0 - 4 days (0-900); Exposure Duration Culture Media: Not determined by stud	dy authors (i)	(U)
Media. Path:	Terrestriar, C	cen culture ivicula, Not determined by sta	ay autions (i.	e., element of interest in exposure water, out unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caer	norhabditis e	legans; wild type; Larvae
Health Outcome:	Behavioral			
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	2215375			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	LOW	Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
· · ·	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Damain 2. Earna ann Ch	····			
Domain 3: Exposure Ch	Matria 7:	Experimental System/Test Media	Uich	The experimental system and methods for momentian of test modio years described in
	Wietric 7.	Preparation	rigii	adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Matria O.	Administration	Low	
	Metric 9:	Concentration	LOW	Exposure concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration tested
		Spacing of Exposure Levels	27/1	
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium
Domain 4. Test Organis	m			
Domain in 1000 organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms
	Metric 15	Conditions Number of Organisms and	Low	The number of test organisms was not reported, repeated tests were used as replicates
	whether 15.	Replicates per Group	Low	(n=3)
Domain 5: Outcome Age	recement			
Domain 5. Outcome As	Metric 16.	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism
		racquies of rest conditions	111511	health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

Diethylhexyl Phthalate

		contin	ued from p	orevious page		
Study Citation:	Tseng, I. L.,	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C.,	V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and		
	AFD neuron	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; (Cell Culture Media; Not determined by stud	y authors (i.	.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; wild type; Larvae		
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.		
Domain 7. Data Present	ation and Anal	lycic				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure related findings were presented for each treatment and control group		
	Wieute 22.	Reporting of Data	mgn	and were adequate to determine values for the endpoint of interest		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	Ascorbic aci	id pretreatment, locomotor and thermotactic	, fig.6			
Overall Qualit	ty Deterr	nination	High			

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Tseng, I. L., AFD neurons Overall Dura Terrestrial; C	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C s through oxidative stress in Caenorhabdit tition: 0 - 4 days (0-96h); Exposure Duratic Cell Culture Media; Not determined by stud Worms (e.g. Annelids Nematodes): Caes	., V.H. (2013) is elegans. PI on: 0 - 4 days dy authors (i.e). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and LoS ONE 8(12):e82657. (0-96h) e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Health Outcome:	Mechanistic-	lechanistic-Neurotoxicology-Ocular and Sensory						
Chemical:	Di-ethylhexy	i-ethylhexyl phthalate (DEHP)						
HERO ID:	2215375	15375						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in				
		Preparation	rug.	adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10.	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type				
	Metric 11:	Number of Exposure Groups/	Low	Only one concentration was tested.				
		Spacing of Exposure Levels						
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.				
Domain 4: Test Organis	m							
6	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, and repeated tests were used as replicates (n=3).				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.				
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.				

Diethylhexyl Phthalate

		contin	ued from p	previous page		
Study Citation:	Tseng, I. L.,	Yang, Y. F., Yu, C. W., Li, W. H., Liao, C.,	V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and		
	AFD neuron	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	y authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis e	elegans; DA 1267; Larvae		
Health Outcome:	Mechanistic	-Neurotoxicology-Ocular and Sensory				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2215375					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
				and were adequate to determine values for the endpoint of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion form is for ascorbic acid pretreatment a	nd AFD the	rmosensory neurons (fig.7).		
Overall Qualit	ty Detern	nination	High			

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Study Citation:	Yin, J., Liu, dependent o	R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wan, ocyte apoptosis and oxidative stress in Caer	g, D. (2018). Di orhabditis elega	(2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage- ns. Ecotoxicology and Environmental Safety 163:298-306.
Duration: Exposure Route, Media Path	Overall Dura Terrestrial; (ation: $\vec{0}$ - $\vec{4}$ days (0-96h); Exposure Duration Cell Culture Media; Not determined by stud	n: 0 - 4 days (0-9 y authors (i.e., ch	(6h) nemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic Di-ethylhexy 4829298	; Worms (e.g., Annelids, Nematodes); <i>Caen</i> -Biomarkers (exposure and effect)-Cytotoxi yl phthalate (DEHP)	orhabditis elegar city-Genotox (in	ns; N2 Wild Type; Larvae cluding DNA repair)
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce	— • • • •	-	
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	I he test substance identity was not analytically verified by the performing laboratory.
	wienie 5.	Test Substance Fullty	LOW	י מוזעי טי צומט טי מול וכא אטאמורל שרול ווטו ובטטונט.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was suitable.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare
	incure /.	Preparation	1011	test concentrations. Concentrations of the test substance were not measured during the study.
	Metric 8:	Consistency of Exposure	Medium	Exposure administration was reported but not in sufficient detail.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration was suitable to determine a dose response.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility, but solvents at an appropriate level aided in disso- lution. The solvent concentration used was not reported, but biological response of the solvent control was adequate.
Domain 4. Test Organis	m			
Domain 4. Test Organis	Metric 13	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and	Low	The number of test replicates was not reported but experiments were repeated.
		Replicates per Group		
Domain 5: Outcome As	sessment			
20mm 5. Outcome Als	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were minimally reported.
		Conti	nued on next pa	ge

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Environmental Hazard Evaluation

HERO ID: 4829298 Table: 1 of 3

		conun	ided from previ	ous page		
Study Citation:	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage- dependent oocyte apoptosis and oxidative stress in Caenorhabditis elegans. Ecotoxicology and Environmental Safety 163:298-306.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)		
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae					
Media, Path:						
Taxa, Species, Age:						
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cytotoxi	city-Genotox (in	cluding DNA repair)		
Chemical:	Di-ethylhexy	yl phthalate (DEHP)	-			
HERO ID:	4829298					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (number of apoptotic cells in oocytes, gene expression of apoptosis and oxidative stress related genes, HUS- 1 protein expression, H2O2 levels and DNA damage) reported the intended outcomes of interest.		
	Metric 18:	Consistency of Outcome Assessment	Medium	There was incomplete reporting of minor details of outcome assessment protocol execu- tion.		
Domain 6: Confounding	/ Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presenta	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group and results were described in the text.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This evaluat	ion form is relevant for all the mechanistic of structure structures and the second structure st	endpoints -numb	er of apoptotic cells in oocytes, gene expression of apoptosis and oxidative stress e in $C_{\rm elegans}$ exposed to 0.1.1 and 10mg/L of DEHP		

Study Citation: Duration: Exposure Route, Media, Path:	Yin, J., Liu, dependent oc Overall Dura Terrestrial; C	R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wan, ocyte apoptosis and oxidative stress in Caer tion: 0 - 4 days (0-96h); Exposure Duration Cell Culture Media; Not determined by stud	g, D. (2018). Di torhabditis elegan h: 0 - 4 days (0-9 y authors (i.e., ch	 (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damagens. Ecotoxicology and Environmental Safety 163:298-306. (6h) memical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caen	orhabditis elegar	<i>is</i> ; N2 Wild Type; Larvae
Health Outcome: Chemical:	Mortality Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	4829298			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce		_	
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Concentrations of test substance were not measured during the study.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure administration was reported but without adequate details. Solvent concentra- tion used was not reported.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	Only a 24 hr exposure but it was suitable to determine non-toxic levels.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable but did not elicit a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility but solvents at an appropriate level aided in dissolu- tion. Biological response of solvent control was adequate.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported but experiments were repeated. Report- ing regarding number of test organisms was confusing. "Thirty L4 nematode larvae were exposed to 24 h for BPA and DEHP at five concentrations of 100, 10, 1, 0.1 and 0.01 mg/L" It was stated that each experiment was repeated three times but data presented only for 30 larvae (Fig 1.).
Domain 5: Outcome As	sessment			

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4829298 Table: 2 of 3

		contir	nued from previ	ous page			
Study Citation:	Yin, J., Liu,	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage- dependent opcyte apoptosis and oxidative stress in Caenorhabditis alegans. Ecotoxicology and Environmental Safety 163:298-306					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route.	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae						
Health Outcome:	Mortality		0				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	4829298						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of test system were minimally reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	Medium	Incomplete reporting of minor details of outcome assessment protocol execution.			
Domain 6: Confounding	/ Variable Co	ntrol					
2 children of Contourioung	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group and results were described in the text. Y-axis label of Fig 1 a) showing mortality data is incorrect.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	No effect of	DEHP on mortality at the concentrations te	sted.				

Study Citation: Duration: Exposure Route, Media, Path: Taxo, Species, Acc:	Yin, J., Liu, J dependent oc Overall Dura Terrestrial; C	 (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damagens. Ecotoxicology and Environmental Safety 163:298-306. 6h) memical of interest in exposure water, but unable to determine exact uptake route) 					
Health Outcome	Reproductive	Vorms (e.g., Annends, Nennatodes), Cuent	ornaballis elegar	is, NZ white Type, Larvae			
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	4829298	· · · · · · · · · · · · · · · · · · ·					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	Source was reported but the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	practarization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Concentrations of test substance were not measured during the study			
	Metric 8:	Consistency of Exposure	Medium	Exposure administration was reported but not in sufficient detail.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration was for 24 hours			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable.			
		Spacing of Exposure Levels	e				
	Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility but solvents at an appropriate level aided in disso- lution. The solvent concentration used was not reported but the biological response of solvent control was adequate.			
Domain 4: Test Organis	m						
-	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported but experiments were repeated. The total number of test organism for each reproductive assay were as follows- 15 nematodes were used for brood size and generation time. 20 nematodes were used to count oocytes, 30 nematodes were used for observing gonadal structure but number of nematodes used per treatment were not provided.			
Domain 5: Outcome Ass	sessment						

PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 4829298 Table: 3 of 3

		contin	ued from previ	ous page			
Study Citation:	Yin, J., Liu, dependent o	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage- dependent oocyte apoptosis and oxidative stress in Caenorhabditis elegans. Ecotoxicology and Environmental Safety 163:298-306.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; N2 Wild Type; Larvae						
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	4829298						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of test system were minimally reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (for brood size, generation time, number of oocytes per gonad arm and observing gonadal structure) reported the intended outcome of interest			
	Metric 18:	Consistency of Outcome	Medium	Incomplete reporting of minor details of outcome assessment protocol execution			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group (Fig 1 and Fig 2) and results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluat exposed to 1	This evaluation form covers a number of reproductive endpoints (brood size, generation time, gonadal structural changes and oocyte count) for C. elegans exposed to 10, 1, and 0.1 mg/L of DEHP.					
Overall Qualit	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	How, C. M., with insulin/ Overall Dura Terrestrial; C Invertebrate; Mortality Di-ethylhexy 5593882	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: > 21 days; Exposure Duration: 4 - 10 days Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae Mortality Di-ethylhexyl phthalate (DEHP) 5593882					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance were not reported.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A sol-			
	Matria 5.	Na antina Cantual Dana ana	TT: -1-	vent control 0.1% DMSO was used.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3. Exposure Ch	aracterization						
Domain J. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."			
	Metric 8:	Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S- basal medium containing E. coli OP50 at 20C for 72 h to reach adult stage."			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (mortality).			
	Metric 11:	Number of Exposure Groups/	N/A	The solvent concentration was appropriate (0.1% DMSO).			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP			
		Testing at or below bolubility Ellilit	mgn	A sorrow was used to emande the solutionty of DEMT.			
Domain 4: Test Organis	m						
if rest organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient.			

Domain 5: Outcome Assessment

Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation: Duration: Exposure Route, Media, Path:	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878. Overall Duration: > 21 days; Exposure Duration: 4 - 10 days Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Invertebrate: Worms (e.g., Annelids, Nematodes): <i>Caenorhabditis elegans</i> : Wild Type N2: Larvae					
Health Outcome:	Mortality	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5593882	5593882					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$. Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."			
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			

strictly informational in nature and could not be used quantitatively in a RE.

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caer	orhabditis el	legans; Wild type Bristol-N2; Larvae				
Health Outcome:	Reproductive/ leratogenic Di-ethylhexyl nhthalate (DEHP)							
HERO ID:	4728405	4728405						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of the compound listed.				
	Metric 2:	Test Substance Source	Low	The source was listed, but the performing lab did not analytically verify the test sub- stance.				
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.				
Domain 2: Test Design								
C C	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.				
	Metric 5:	Negative Control Response	High	Control responses for total progeny per day within the 5-day study are reported in Figure 4A.				
	Metric 6:	Randomized Allocation	Low	No random allocation was described.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure on	Metric 7:	Experimental System/Test Media	High	Test media preparation was described adequately in section 2.1.				
		Preparation	0	I I I I I I I I I I I I I I I I I I I				
	Metric 8:	Consistency of Exposure	Medium	The one treatment concentration appeared to be administered similarly.				
	Metric 9:	Administration Measurement of Test Substance	Low	No compounds were measured for analytical verification of treatment concentrations,				
	M (10	Concentration	TT' 1	and concentrations were reported as nominal.				
	Metric 10:	Exposure Duration and Frequency	High	The study only examined are treatment concentration (1.10). No instifaction are				
	Metric 11:	Specing of Exposure Levels	LOW	I ne study only examined one treatment concentration (1 uM). No justification was given for just using a single concentration				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for C. elegans is a solid.				
Domain 4: Test Organis	m							
• 1000 01gamo	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment and section 2.5 details the handling of animals for fecundity. Rearing media before and during testing was the same (minus the compound).				
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was one animal per plate and 24 animals for the treatment concentration and corresponding control.				

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

HERO ID: 4728405 Table: 1 of 1

radhan, A., (Olegon D.E. Logg I (2018) Di(2 othershop				
Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.					
verall Durat	tion: 4 - 10 days; Exposure Duration: 4 - 1	0 days			
Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Wild type Bristol-N2; Larvae					
eproductive	/Teratogenic				
i-ethylhexy	phthalate (DEHP)				
728405					
	Metric	Rating	Comments		
letric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.		
fetric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.		
fetric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.		
ariable Con	trol				
letric 19:	Confounding Variables in Test	High	There were no reported differences that would indicate confounding variables.		
	Design and Procedures	8			
letric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.		
n and Analy	reie				
letric 21.	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc		
letric 21.	Reporting of Data	High	The presentation of total progeny per day is presented in figure 4A		
Ietric 22:	Explanation of Unexpected Outcomes	Low	Scatter plots are used for total progeny, so no variance terms are presented.		
The study only examined one treatment concentration (1 uM) and exposure concentration was not measured. No justification was given for using a single concentration. DEHP reduced the fecundity at 1 mM concentration.					
	verali Durat errestrial; Convertebrate; eproductive i-ethylhexyl 728405 letric 16: letric 17: letric 17: letric 18: letric 18: letric 19: letric 20: letric 20: n and Analy letric 21: letric 22: letric 23: he study onloncentration	Verali Duration: 4 - 10 days; Exposure Duration: 4 - 1 errestrial; Cell Culture Media; Not determined by stud evertebrate; Worms (e.g., Annelids, Nematodes); <i>Caen</i> eproductive/Teratogenic i-ethylhexyl phthalate (DEHP) 728405 <u>Metric</u> letric 16: Adequacy of Test Conditions letric 17: Outcome Assessment Methodology letric 18: Consistency of Outcome <u>Assessment</u> Variable Control letric 19: Confounding Variables in Test Design and Procedures letric 20: Outcomes Unrelated to Exposure n and Analysis letric 21: Statistical Methods letric 23: Explanation of Unexpected Outcomes he study only examined one treatment concentration (oncentration. DEHP reduced the fecundity at 1 mM co	Verali Duration: 4 - 10 days; Exposure Duration: 4 - 10 days errestrial; Cell Culture Media; Not determined by study authors (i.e. evertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis ele eproductive/Teratogenic i-ethylhexyl phthalate (DEHP) 728405 Metric Rating Ietric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Methodology High Ietric 18: Consistency of Outcome High Zariable Control Ietric 20: Outcomes Unrelated to Exposure Medium n and Analysis Ietric 21: Statistical Methods Ietric 23: Explanation of Unexpected Outcomes Low he study only examined one treatment concentration (1 uM) and explanation.		

May 2025

Study Citation: Duration: Exposure Route, Modio, Both	Pradhan, A., of Caenorhal Overall Dura Terrestrial; C	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Wild type Bristol-N2; Larvae						
Health Outcome:	Other (please specify below) (Lifespan)							
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	4728405							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.				
	Metric 2:	Test Substance Source	Low	Source was listed, but the performing lab did not analytically verify the test substance.				
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.				
	Metric 5:	Negative Control Response	Medium	Control responses for lifespan were reported in the narrative as 14 days on page 6/8. No variance in this metric was reported.				
	Metric 6:	Randomized Allocation	Low	No random allocation was described.				
Domain 3: Exposure Ch	aracterization		TT' 1					
	Metric /:	Preparation	High	lest media preparation was described adequately in section 2.1.				
	Metric 8:	Consistency of Exposure	Medium	The one treatment concentration appeared to be administered similarly.				
		Administration		The one dominant concentration appeared to be administered similarly.				
	Metric 9:	Measurement of Test Substance	Low	No compounds were measured for analytical verification of treatment concentrations,				
	Matria 10.	Concentration	II: -1	and concentrations were reported as nominal.				
	Metric 10:	Exposure Duration and Frequency	High	The 20 day duration for lifespan seems to be appropriate.				
	Meure II:	Spacing of Exposure Levels	LOW	for just using a single concentration				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for C. elegans is a solid.				
		6		<i>c c c</i>				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment and section 2.5 details the handling of animals for lifespan. Rearing media before and during testing was the same (minus the compound).				
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was one animal per plate and 24 animals for the treatment concentration and corresponding control.				

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 4728405 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation:	Pradhan, A., of Caenorha	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Worms (e.g., Annelids, Nematodes); Caeno	orhabditis elegai	as; Wild type Bristol-N2; Larvae			
Health Outcome:	Other (pleas	e specify below) (Lifespan)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	4728405						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.			
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.			
	Metric 18:	Consistency of Outcome	High	The outcome assessment appeared to be assessed consistently among treatment groups.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences that would indicate confounding variables.			
		Design and Procedures	U				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would inter- fere with the results.			
Domain 7: Data Present	ation and Anal	lucio					
Domanii 7. Data Present	Metric 21:	Statistical Methods	High	Authors used a Mantel-Cox test to determine if lifespan was significantly different from control lifespan. (page 6/8).			
	Metric 22:	Reporting of Data	Medium	The presentation of survival (lifespan) is presented in figure 4B.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variance terms for lifespan were reported.			
Additional Comments:	The study or DEHP reduc	The study only examined one treatment concentration (1 uM). No justification was given for using a single concentration. Lifespan analysis indicated that DEHP reduced the average lifespan from 14 days in unexposed worms to 13 days in exposed worms.					
Overall Quali	ty Deterr	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: Not-reported; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton-S strain; Adult Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP)				
Demain	11/84019	N. f. e: -	Detine	Commente	
Domain Domain 1: Test Substand	20	Metric	Rating	Comments	
Domain 1. Test Substant	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was identified by CASRN as seen in Fig. 1. The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.	
	Metric 5:	Negative Control Response	High	The control responses of the fertility test were reported in Fig. 4 and were adequate for the outcome of interest.	
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.	
Domain 3: Exposure Cha	aracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. Study authors reported 20 brand-new flies were taken from the standard medium and put into tubes with the test solution. The number of pairs per tube was unclear.	
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.	
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the fertility test was reported to be three days. The flies were then removed from tubes with the test material so females could mate and lay eggs. The test was ended after pupae and eclosion were determined. This appeared adequate for the outcome of interest.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only two exposure levels. This allowed for a comparison between a low and a high exposure concentration, but more exposure levels may have yielded more data.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via diet.	

Environmental Hazard Evaluation

		contin	ued from previ	ious page	
Study Citation:	Liu, X., Li, on AKT/FO	X., Liu, Y., Wu, W. D., Liu, X. M. (2024). 1 XO pathway. Toxicology In Vitro 95:105742	DEHP and DIN	P accelerate aging effects in male and female of Drosophila melanogaster depend	
Duration:	Overall Dur	ation: Not-reported; Exposure Duration: 0 -	4 days (0-96h)		
Exposure Route,	Terrestrial;	Food/Diet; Dietary			
Media, Path:					
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; Car	nton-S strain; Ac	dult	
Health Outcome:	Reproductiv	re/Teratogenic			
Chemical:	Di-ethylhexyl phthalate (DEHP)				
HERO ID:	11784619				
Domain		Metric	Rating	Comments	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The Canton-S strain fruit flies were from Core Facility of Drosophila Resource and Technology at the CAS Center for Excellence in Molecular Cell Science. The flies were reported to be three days old at the start of the test.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 20 pairs of flies used in the fertility test. It was unclear if this was 20 pairs per treatment or 20 pairs total. Authors reported each treatment was repeated four times.	
Domain 5: Outcome Ag	aggment				
Domain 5. Outcome As	Matria 16	A deguage of Test Conditions	Law	Tista dataila anno anno idad an sha antonina ann disiana and sha saas sadistana	
	Metric 10:	Adequacy of Test Conditions	LOW	The details were provided on the culturing conditions and the test conditions.	
	Metric 17:	Outcome Assessment Methodology	High	interest-the number of pupae and eclosions after exposure.	
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Flies were exposed for three days and then removed from the test chemical. Females were allowed to lay eggs, and the number of pupae and egg eclosions were monitored.	
Domain 6: Confounding	/ Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental	
		Design and Procedures		conditions or other non-treatment-related factors across study groups.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Present	ation and Ana	lvsis			
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.	
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the reproductive tests were reported in Fig. 4 and were adequate for the outcome of interest.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.	
Additional Comments:	This portion They were e diet.	of the evaluation is for the reproductive experimentary exposed for three days after which, the number	osure to DEHP. per of egg eclos	D. melanogaster were exposed to DEHP at two concentrations of 1uM and 10uM. ions was determined. There were also different diets, a normal diet and a high fat	
Overall Qualit	y Deterr	nination	Medium	l	

Overall Quality Determination
PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 11784619 Table: 1 of 2

... continued from previous page **Study Citation:** Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. **Duration:** Overall Duration: Not-reported; Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Terrestrial; Food/Diet; Dietary Media, Path: Invertebrate; Arthropods; Drosophila melanogaster; Canton-S strain; Adult Taxa, Species, Age: **Health Outcome:** Reproductive/Teratogenic Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 11784619 Domain Metric Rating Comments

Study Citation:	Liu, X., Li, on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.					
Duration: Exposure Route, Media. Path:	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Mortality Di-ethylhexy	; Arthropods; <i>Drosophila melanogaster</i> ; Ca yl phthalate (DEHP)	nton-S strain; Ad	ult			
HERO ID:	11784619	-					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Motria 1:	Tast Substance Identity	Uiah	The test substance use identified by CASDN as seen in Fig. 1			
	Metric 2:	Test Substance Source	Low	The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Tast Dasign							
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the acute tests were reported in Fig. 2 and were adequate for the outcome of interest. The control responses of the high fat diet organisms were also reported in Table 2.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domani 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the acute tests was reported to be 24h. The flies were then assessed for lifespan after this exposure. This appeared adequate to assess the desired outcomes.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only two exposure levels. This allowed for a comparison between a low and a high exposure concentration, but more exposure levels may have yielded more data.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						

Environmental Hazard Evaluation

HERO ID: 11784619 Table: 2 of 2

		conti	nued from previ	ous page				
Study Citation:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.							
Duration:	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h)							
Exposure Route,	Terrestrial; Food/Diet; Dietary							
Media, Path:								
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton-S strain; Adult							
Health Outcome:	Mortality							
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	11784619	-						
Domain		Metric	Rating	Comments				
	Metric 13:	Test Organism Characteristics	High	The Canton-S strain fruit flies were from Core Facility of Drosophila Resource and Technology at the CAS Center for Excellence in Molecular Cell Science. The flies were reported to be three days old at the start of the test.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the flies were acclimated prior to the start of the study.				
	Metric 15:	Conditions Number of Organisms and	Medium	It was reported that there were 80-100 flies per group with three replicates each for the				
		Replicates per Group						
Domain 5: Outcome As	ssessment							
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan after a 24h exposure (mortality).				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for 24h. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet every week until all flies had died.				
Domain 6: Confoundin	g / Variable Co	ntroi Confounding Variables in Test	Low					
	Metric 19:	Design and Procedures	LOW	conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7. Data Presen	tation and Anal	vsis						
Domain 7. Data Heseli	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of				
	Metric 22:	Reporting of Data	High	Interest. The responses for the controls and the exposure groups for the acutes tests were reported in Fig. 2 and were adequate for the outcome of interest. Table 2 has data for the HFD				
	Metric 23:	Explanation of Unexpected Outcomes	High	oniy. Study authors did not report any unexpected outcomes.				
Additional Comments:	This portion 10uM. They	of the evaluation is for the acute exposure t were exposed for 24h and then lifespan wa	o DEHP. Three-o	lay old D. melanogaster were exposed to DEHP at two concentrations of 1uM and e were also different diets, a normal diet and a high fat diet.				

Study Citation:	Cao, H., Wie in Drosophil Overall Dura	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in Drosophila melanogaster. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316. Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days						
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild-type; Embryo						
Health Outcome:	Behavioral	d abtholoto (DEUD)						
HERO ID.	5495570	(DEHP)						
Domain	515570	Metric	Rating	Comments				
Domain 1: Test Substan	ce	incure	Runng	Commonas				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Low	Reporting omissions could have a substantial impact on results.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was not clearly reported.				
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure concentration with an appropriate solvent concentration.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Number of Organisms and	Low	The number of replicates was not reported.				
		Replicates per Group		· ·				
Domain 5: Outcome Ass	recoment							
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if adequate				
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest.				
		Contir	und on novt no	πΔ				
Continued on fiext page								

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in Drosophila melanogaster. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Exposure Route, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild-type; Embryo **Health Outcome:** Behavioral Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 5495570 Domain Metric Rating Comments Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: High There were no reported differences in environmental conditions or other factors. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium No information was presented to suggest differences in animal attrition or health outcomes that would impact results. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Statistical analysis was performed but not described adequately. High Metric 22: Reporting of Data Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes High Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation is for food preference, CAFE, activity, and sleeping index.

Overall Quality Determination

Medium

HERO ID: 5495570 Table: 2 of 3

Study Citation:	Cao, H., Wie	emerslage, L., Marttila, P. S., Williams, M. J a melanogaster, Basic & Clinical Pharmaco	J., Schiöth, H. B.	(2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels asy Online Pharmacology Online 119(3):309-316		
Duration: Exposure Route, Media Path:	Overall Dura Terrestrial; F	ation: 4 - 10 days; Exposure Duration: 4 - 16 Food/Diet; Dietary	0 days			
Taxa, Species, Age:	Invertebrate:	Arthropods; <i>Drosophila melanogaster</i> ; CS	ORC wild-type;	Embryo		
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)	0			
HERO ID:	5495570					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
Domain 2: Exposure Ch	aractorization					
Domain 5. Exposure Ch	Matria 7	Experimental System/Test Media	Low	The study merided only limited details on the macquires taken to enmonaistaly menors		
	Metho 7.	Preparation	LOW	test concentrations		
	Metric 8:	Consistency of Exposure	Low	Reporting omissions could have a substantial impact on results		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Matria 10	Concentration	Law			
	Metric 10:	Exposure Duration and Frequency	LOW	The number of exposure was not clearly reported		
	Metric 11:	Spacing of Exposure Levels	nign	response		
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via diet		
Domain 1: Test Organis	m					
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms,		
		Conditions	2			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of replicates was not reported.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if adequate		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest		
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups		
Domain 6: Confounding	/ Variable Co	ntrol				

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5495570 Table: 2 of 3

		contir	nued from previo	us page		
Study Citation:	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in Drosophila melanogaster. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.					
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild-type; Embryo					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5495570					
Domain		Metric	Rating	Comments		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing reported to indicate that environmental conditions or other factors influenced outcome assessment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained		
Additional Comments:	Gene expres	sion and circulating lipid/carbohydrate				
Overall Qualit	ty Detern	nination	Medium			

Study Citation:	Cao, H., Wi	emerslage, L., Marttila, P. S., Williams, M. J la melanogaster. Basic & Clinical Pharmaco	J., Schiöth, H. B.	. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels by Online Pharmacology Online 119(3):309-316.				
Duration: Exposure Route, Madia Bath	Overall Dur Terrestrial; I	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo Mortality						
Tava Species Age	Invertebrate							
Health Outcome:	Mortality							
Chemical:	Di-ethylhex	yl phthalate (DEHP)						
HERO ID:	5495570							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
2 children en 24 pessare en	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Low	Reporting omissions could have a substantial impact on results.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was not clearly reported				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate.				
		Spacing of Exposure Levels	8					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Test Organis	m							
U	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of replicates was not reported.				
Domain 5: Outcome A -	agamant							
Domain 5. Outcome As	Metric 16	A dequacy of Test Conditions	Low	Deporting of environmental conditions was not sufficient to avaluate if adapted				
	Metric 17:	Autquaty of Test Collutions Outcome Assessment Methodology	LOW Medium	The outcome assessment methodology reported the intended outcome of interest				
	Metric 18	Consistency of Outcome	High	Outcomes were assessed consistently across study groups				
		Assessment	Ingii	oucomes were assessed consistently across study groups.				
Domain 6: Confounding	g / Variable Co	ntrol						

		contin	ued from previ	ous page			
Study Citation:	Cao, H., Wie in Drosophil	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schlöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in Drosophila melanogaster. Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316					
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days						
Exposure Route,	Terrestrial; Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild-type; Embryo						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5495570						
Domain		Metric	Rating	Comments			
	Metric 19:	Confounding Variables in Test	High	No differences in environmental conditions or other non treatment conditions/factors			
		Design and Procedures		were reported.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors did not report if there were no differences among groups			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained			
Additional Comments:	starvation re-	sistance-presented in terms of survival hour	s in Figure 1, thu	us the mortality endpoint.			
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Chen, M. Y.	., Liu, H. P., Cheng, J., Chiang, S. Y., Li	ao, W. P., Lii	n, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila.			
	Chemospher	Chemosphere 221:493-499.					
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days						
Exposure Route, Modia Dath	Terrestrial; Food/Diet; Dietary						
Tava Species Age	Invertebrate	Arthropods: Drosonhila melanogaster: A	dult				
Health Outcome	Reproductiv	e/Teratogenic	uun				
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	5495717	(PEIII)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Identified by CASRN 117-81-7			
	Metric 2:	Test Substance Source	High	Sourced from Alfa Aesar			
	Metric 3:	Test Substance Purity	High	Purity given as "98+%"			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative control used.			
	Metric 5:	Negative Control Response	High	No unacceptable responses in control.			
	Metric 6:	Randomized Allocation	Medium	Authors reported randomized allocation.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	Media preparation was detailed & adequate.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and were only given as percentages (pre- sumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administrated media. If it is assumed that the percentages given are wet weights of total culture medium, the concentrations can be back-calculated from the given volumes and masses of constituents, but this is not certain.			
	Metric 10:	Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose- response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via diet.			
Domain 4: Test Organis	m	T A A A					
	Metric 13:	Test Organism Characteristics	Medium	Wild-type D. melanogaster of uncertain provenance.			
	Metric 14:	Acclimatization and Pretreatment	Low	No report of acclimatization.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	30-35 flies per replicate, number of replicates not reported.			
		Cont	inued on nex	t page			

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Environmental Hazard Evaluation

HERO ID: 5495717 Table: 1 of 1

Liu, H. P., Cheng, J., Chiang, S. Y., Lia 221:493-499. on: 4 - 10 days; Exposure Duration: 4 - 1 od/Diet; Dietary arthropods; <i>Drosophila melanogaster</i> ; Ac Feratogenic phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions	ao, W. P., Li 10 days dult Rating	n, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila.
Adequacy of Test Conditions	10 days dult Rating	Comments
od/Diet; Dietary Arthropods; <i>Drosophila melanogaster</i> ; Ac Feratogenic phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions	dult Rating	Comments
Arthropods; <i>Drosophila melanogaster</i> ; Ac Teratogenic phthalate (DEHP) Metric Adequacy of Test Conditions	dult Rating	Comments
Arthropods; <i>Drosophila melanogaster</i> ; Ac Teratogenic phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions	Rating	Comments
Teratogenic phthalate (DEHP) Metric Adequacy of Test Conditions	Rating	Comments
hthalate (DEHP) Metric Adequacy of Test Conditions	Rating	Comments
Metric Adequacy of Test Conditions	Rating	Comments
Metric Adequacy of Test Conditions	Rating	Comments
Adequacy of Test Conditions	T	
Adequacy of Test Conditions	τ	
	Low	Reporting insufficient to determine test conditions (only nominal temperature & pho- toperiod given).
Outcome Assessment Methodology	Medium	Flies weighed in batches of 10 under anesthesia.
Consistency of Outcome	High	Outcomes assessed consistently across groups.
Assessment	-	
rol		
Confounding Variables in Test	High	No confounding variables reported.
Design and Procedures	C C	
Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure reported.
is		
Statistical Methods	High	One-way ANOVA tables provided.
Reporting of Data	High	Data reported for all outcomes.
Explanation of Unexpected Outcomes	High	No unexplained outcomes reported.
was not reported & cannot be back-calcu	lated with co	onfidence from the given information, this study is low-quality.
	Outcome Assessment Methodology Consistency of Outcome Assessment ol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure is Statistical Methods Reporting of Data Explanation of Unexpected Outcomes vas not reported & cannot be back-calcu	Outcome Assessment Methodology Consistency of Outcome Medium High Assessment High ol Image: Confounding Variables in Test High Design and Procedures Medium Outcomes Unrelated to Exposure Medium is Statistical Methods High Explanation of Unexpected Outcomes High was not reported & cannot be back-calculated with comparison Low

Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.					
Duration: Exposure Route, Media, Path:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; O	Canton Special	; Adult		
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5494836					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).		
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.		
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.		
	Metric 5:	Negative Control Response	High	Re-mating assay: Based on the description and results by the authors the biological response of the control group (re-mating rate of normal females with DEHP-untreated males, Fig 8B) was adequate.		
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation in the re-mating assay.		
Demain 2: Error Ch						
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% \text{ v/v}$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.		
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirm DEHP was dispersed after mixing, culture medium was examined by microscopy.		
	Metric 10:	Exposure Duration and Frequency	High	In Section 2.8, the authors reported that virgin flies were separated by sex and treated with 0, 0.1, 0.2, and 0.4% DEHP respectively, and that at day 10, the mating assays were performed. A 10-day exposure appeared adequate to study the response.		
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Diethylhexyl Phthalate

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Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route,	Terrestrial; Food/Diet; Dietary
Media, Path:	
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	5494836

Domain	Metric	Rating	Comments
Metric 11: Metric 12:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit	High Medium	Re-mating assay: DEHP exposure groups were Ctrl (0%), 0.1, 0.2, and 0.4% DEHP as described in Section 2.8. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016). DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal
			concentrations were to actual concentrations.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.
Metric 15:	Number of Organisms and Replicates per Group	Low	In Section 2.8, the authors reported using 24 replicates of naïve virgin female flies to mate with each DEHP-treated male one by one. However, it is not clear how many males they included in the study. Also, did they mean 24 female flies per treatment group? That is, it is unclear how many female and male flies were per group and how many treatment replicates there were.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (re-mating assay) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on reproductive system of males).
Metric 18:	Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treat- ment groups.
Domain 6: Confounding / Variable Co	ntrol		
Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
	Design and Procedures	2	that would influence the outcome.
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Diethylhexyl Phthalate

		contil	nued from p	revious page			
Study Citation:	Chen, M. Y. junction, and	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult					
Health Outcome:	Reproductive	Reproductive/Teratogenic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5494836						
Domain		Metric	Rating	Comments			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 8B).			
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figure 8B and Supplementary Table 11.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.			
Additional Comments:	Summary no melanogaster and (4) on r	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysylology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by					

and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscu junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary							
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Neurological Di-ethylhexy 5494836	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Larvae Neurological Di-ethylhexyl phthalate (DEHP) 5494836						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).				
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.				
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.				
	Metric 5:	Negative Control Response	High	Electrophysiology of Drosophila third-instar larvae neuromuscular junction: the biolog- ical response is expected to be adequate given the evidence in methodology provided by the authors.				
	Metric 6:	Randomized Allocation	Low	The study did not specifically state 'random allocation' or how organisms were allocated for the electrophysiological assessment of the neuromuscular junction in Drosophila larvae.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% v/v$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.				
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consis- tently across study groups.				
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.				
	Metric 10:	Exposure Duration and Frequency	High	Exposure of 5 days was adequate to assess the electrophysiology of the neuromuscular junction as described by the authors.				
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Diethylhexyl Phthalate

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Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route,	Terrestrial; Food/Diet; Dietary
Media, Path:	
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Larvae
Health Outcome:	Neurological
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	5494836

Domain	Metric	Rating	Comments		
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Electrophysiological assessment of the neuromuscular junction: DEHP exposure groups were Ctrl (0%), 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).		
Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.		
Domain 4: Test Organism					
Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.		
Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.		
Metric 15:	Number of Organisms and Replicates per Group	Low	Authors reported 11 (Control), 9 (0.2% DEHP), or 6 (0.4% DEHP) flies per treatment group in Figure 4, but they did not use treatment replicates per group. The number of flies in the high dose group was almost half of that in the control group.		
Domain 5: Outcome Assessment					
Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.		
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (electrophysiology of the neuromuscular junc- tion) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on neural transmission).		
Metric 18:	Consistency of Outcome Assessment	High	Method details of the electrophysiology of the neuromuscular junction to evaluate neural transmission were reported and assessed consistently across study groups.		
Domain 6: Confounding / Variable Co	ntrol				
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.		
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.		
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Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Chen, M. Y. junction, and Overall Dura Terrestrial; F Invertebrate: Neurologica Di-ethylhex	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Larvae Neurological Di-ethylhexyl phthalate (DEHP)				
Domain	3494830	Metric	Rating	Comments		
Domain 7: Data Present Additional Comments:	Itation and Analysis Metric 21: Statistical Methods High Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for the post-hoc test (Figure 4). Metric 22: Reporting of Data High Data was reported for all treatment group the text and Figure 4 and Supplementary Tables 7 and 8. Metric 23: Explanation of Unexpected Outcomes High Authors reported the standard error of the mean. Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosoph					
	melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.					

Overall Quality Determination



Study Citation: Duration: Exposure Route, Media. Path:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic Di-ethylhexy 5494836	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5494836					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).			
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.			
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.			
	Metric 5:	Negative Control Response	High	Gene expression in reproductive system of male Drosophila: Based on the description and results by the authors the biological response of the control group (mRNA expres- sion of genes encoding proteins of the reproductive system) was adequate.			
	Metric 6:	Randomized Allocation	Medium	The authors indicated in Section 3.5 that Drosophila, male flies after eclosion were ran- domly divided into groups, and continuously supplied with culture medium containing a series of concentrations of DEHP respectively for 7 days.			
Domain 3: Exposure Ch	aracterization						
·	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% \text{ v/v}$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.			
	Metric 8:	Consistency of Exposure	High	There is no evidence showing that exposure administration was not administered consis- tently across study groups			
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.			
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Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route, Media Path:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5494836						
Domain		Metric	Rating	Comments			
	Metric 10:	Exposure Duration and Frequency	High	The authors reported in Section 3.5 that Drosophila, male flies after eclosion were ran- domly divided into groups, and continuously supplied with culture medium containing a series of concentrations of DEHP respectively for 7 days. A 7 day exposure is adequate to capture changes in gene expression.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Gene expression in reproductive system of male Drosophila: DEHP exposure groups were Ctrl (0%), 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).			
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.			
Domain 4. Test Organis	m						
_ sham is rost organis	Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Gene expression in reproductive system of male Drosophila: The number of test organ- isms and replicates was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.			
	Metric 17:	Outcome Assessment Methodology	High	The methodology used to measure mRNA expression (qPCR) was sufficiently described.			
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treat- ment groups.			
Domain 6: Confounding	o / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.			
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Diethylhexyl Phthalate

		contir	nued from p	revious page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 5494836				
Domain		Metric	Rating	Comments	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 8A).	
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High High	Data was reported for all treatment group as per Figure 8A and Supplementary Table 10. Authors reported the standard error of the mean.	
Additional Comments:	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.				

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Vogel, E. W.,	/ogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombina-							
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 4 - 10 days							
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:									
Taxa, Species, Age: Health Outcome:	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; y female x w male, Leiden Strain; Embryo								
Chemical:	Di-ethylhexy	l phthalate (DEHP)							
HERO ID:	200657								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN in Table VI.					
	Metric 2:	Test Substance Source	Low	The DEHP was only reported to be commercially available; the source was not reported, nor was it reported to be analytically verified.					
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DEHP was not reported.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	The study authors reported using a concurrent negative control as seen in Table VI. The solvents used were 3% ethanol and 1% Tween.					
	Metric 5:	Negative Control Response	High	The negative control response for DEHP was reported in Table VI and was adequate for the outcome of interest. All control results from unexposed larvae in all the exposures were reported in Table 1. The C-1 Eth./T control and pooled control results from all C-1 tests were reported here.					
	Metric 6:	Randomized Allocation	Low	It was not reported how the Drosophila were allocated into study groups.					
	, · ,·								
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	I imited details were provided on the propagation of the test concentrations and on the					
	Metric 7.	Preparation	Low	test system. It was reported that the test for DEHP was a chronic test in which 12-15 adult flies were placed in bottles and permitted to lay eggs for three days on food supple- mented with the test substance dissolved in solvent before mixing into the standard food. Newly hatched females were transferred to fresh medium and scored 1-5 days later. Eyes of adult females were inspected for mosaic light spots under a dissecting scope.					
	Metric 8:	Consistency of Exposure Administration	Low	Limited details were provided on the exposure system and the preparation of the test substance, so reporting omissions prevented a determination on consistency.					
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured at any point in the study.					
	Metric 10:	Exposure Duration and Frequency	Low	The exact exposure duration was not reported. The chronic test reported that the adult flies were allowed to lay eggs onto treated media for three days. Newly hatched females were then transferred to fresh medium and their eyes were scored for mosaics 2-5 days later.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only two exposure groups, 10mM and 20mM, which is lower than is typical. Neither concentration reported a response.					
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via food, though the exposure could also have been via contact with the test substance in the food.					

Diethylhexyl Phthalate

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Study Citation:	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombina- tion. Mutagenesis 8(1):57-81.									
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 4 - 10 days							
Exposure Route, Media. Path:	Terrestrial; F	Food/Diet; Not determined by study authors	s (i.e., chemical o	f interest in exposure water, but unable to determine exact uptake route)						
Taxa. Species. Age:	Invertebrate:	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; y female x w male, Leiden Strain; Embryo Mechanistic-Genotox (including DNA repair)								
Health Outcome:	Mechanistic									
Chemical:	Di-ethylhexy	vl phthalate (DEHP)								
HERO ID:	200657	(- p)								
Domain		Metric	Rating	Comments						
Domain 4: Test Organi	ism									
	Metric 13:	Test Organism Characteristics	Low	The source of the Drosophila was not reported. It was reported strains y and w were from two different laboratory stocks, but it was unclear if those were cultured in house.						
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated to test conditions.						
	Metric 15:	Number of Organisms and	Low	The number of eves for the control and both the exposure groups was reported in Table						
		Replicates per Group		VI. However, the number of replicates per exposure group was not reported.						
Domain 5: Outcome A	ssessment									
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions of the Drosophila. It is unclear what the temperature was and how large the exposure vessels were. They were fed, but the amount of food was not reported.						
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-the number of adult female Drosophila that had mosaic eyes due to genotoxicity in comparison to the controls. Mosaic spots were classified according to size class, and analysis of clone size and distribution was reported.						
	Metric 18:	Consistency of Outcome Assessment	Medium	It was reported that female Drosophila were assessed for mosaics 1-5 days after they were transferred to fresh media after they had hatched. This creates some inconsistency in when they were assessed, which may be due to when the eggs were oviposited. It was unlikely to have any substantial effect on the results.						
Domain 6: Confoundir	ng / Variable Cor	ntrol								
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Little information was provided on environmental conditions, nor was it reported if the organisms were acclimated.						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.						
Domain 7: Data Preser	ntation and Anal	vsis								
	Metric 21:	Statistical Methods	High	A distinction was made regarding the size of the spots, and the total spots was reported. The frequency of clones per 10 ⁴ cells was calculated according to the formula f=2nm/NC, which is described in the text. The LED and HDT were calculated for active and inactive chemicals. A Chi-square test was reported to be used as statistical evaluation and test responses were categorized into strong responses, weakly positive responses, and negative responses.						
	Metric 22:	Reporting of Data	High	Data for the DEHP exposure and the DEHP control were reported in Table VI, which was the inactive chemical table. The results were adequate for the outcome of interest.						

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All control results for each test condition were provided in Table I.

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		continu	ued from previ	ous page		
Study Citation:	Vogel, E. W	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombina-				
	tion. Mutage	enesis 8(1):57-81.	4 10 1			
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	4 - 10 days			
Exposure Route,	Terrestrial; I	Food/Diet; Not determined by study authors (i.e., chemical o	f interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate: Arthropods; Drosophila melanogaster: y female x w male, Leiden Strain; Embryo					
Health Outcome:	Mechanistic-Genotox (including DNA repair)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	200657					
Domain		Metric	Rating	Comments		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not provide any measures of variability even though average clone size and clones per 10^4 cells were reported.		
Additional Comments:	onal Comments: This evaluation was on the effect of DEHP on genetic recombination in Drosophila. In the DEHP test, y males were crossed with w females and allowed to oviposit in bottles containing food supplemented with the test substance. Freshly hatched females were then transferred to fresh media and their eyes were assessed for mosaic spots 1-5 days later. Mechanistic genotoxicity was selected as the outcome of interest.					
Overall Quali	ty Deterr	nination	Medium			

Study Citation:	Chen, M. Y.	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila.						
Duration:	Chemospher Overall Dura	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	; Arthropods; <i>Drosophila melanogaster</i> ; A	dult					
Chemical:	Di-ethylbey	vl phthalate (DEHP)						
HERO ID:	5495717	(PEIII)						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN (117-81-7).				
	Metric 2:	Test Substance Source	High	The test substance was sourced from Alfa Aesar.				
	Metric 3:	Test Substance Purity	High	Authors reported the purity as "98+%."				
Domain 2: Test Design								
c c	Metric 4:	Negative Controls	High	A negative control was used.				
	Metric 5:	Negative Control Response	High	There were no unacceptable responses in the control.				
	Metric 6:	Randomized Allocation	Medium	Authors reported randomized allocation.				
Domain 3: Exposure Ch	aracterization							
I I I I I I I I I I I I I I I I I I I	Metric 7:	Experimental System/Test Media	High	Media preparation was detailed & adequate.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and were only given as percentages (pre- sumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administrated media. If it is assumed that the percentages given are wet weights of the total culture medium. The concentra- tions can be back-calculated from the given volumes and masses of constituents, but this is not certain.				
	Metric 10:	Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose- response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Test Organis	m Matria 12	Test Organism Characteristics	Madium					
	Metric 14:	Acclimatization and Pretreatment	Low	The study used while type D. metanogaster of uncertain provenance.				
	1010uie 17.	Conditions	LOW	There was no report of accimianzation.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 30-35 flies per replicate, but the number of replicates was not reported.				
Continued on next page								

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 5495717 Table: 1 of 2

		conti	nued from p	revious page				
Study Citation:	Chen, M. Y. Chemospher	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila. Chemosphere 221:493-499.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Terrestrial; F	Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; Ac	dult					
Health Outcome:	Reproductiv	e/Teratogenic						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5495717							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Reporting was insufficient to determine test conditions (only nominal temperature & photoperiod given).				
	Metric 17:	Outcome Assessment Methodology	Medium	Emergence (reproductive success) was evaluated after five days.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.				
Domain 7: Data Present	ation and Anal	veis						
Domain 7. Dua Present	Metric 21	Statistical Methods	High	One-way ANOVA tables were provided				
	Metric 22:	Reporting of Data	High	Data were reported for all outcomes				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported.				
Additional Comments:	Because dos	e was not reported & cannot be back-calcu	lated with co	nfidence from the given information, this study is low-quality.				
Overall Qualit	ty Detern	nination	Low					

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HEPO ID:	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila. Chemosphere 221:493-499. Overall Duration: > 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult Mortality Di-ethylhexyl phthalate (DEHP)					
Domain	5495717	Metric	Rating	Comments		
Domain 1: Test Substan	ce.	Wiette	Rating	connients		
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	High High High	Identified by CASRN 117-81-7 Sourced from Alfa Aesar Purity given as "98+%"		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Negative control used.		
	Metric 5:	Negative Control Response	High	No unacceptable responses in control.		
	Metric 6:	Randomized Allocation	Medium	Authors reported randomized allocation.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	High	Media preparation was detailed & adequate.		
	Metric 8:	Preparation Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and were only given as percentages (pre- sumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administrated media. If it is assumed that the percentages given are wet weights of total culture medium, the concentrations can be back-calculated from the given volumes and masses of constituents, but this is not certain.		
	Metric 10:	Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose- response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via diet.		
Domain 4: Test Organis	m					
c	Metric 13:	Test Organism Characteristics	Medium	Wild-type D. melanogaster of uncertain provenance.		
	Metric 14:	Acclimatization and Pretreatment	Low	No report of acclimatization.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	30-35 flies per replicate, number of replicates not reported.		

Domain 5: Outcome Assessment

HERO ID: 5495717 Table: 2 of 2

		conti	nued from p	revious page				
Study Citation:	Chen, M. Y. Chemospher	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of Drosophila. Chemosphere 221:493-499.						
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days							
Exposure Route,	Terrestrial; F	Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	; Arthropods; Drosophila melanogaster; A	dult					
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5495717							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting insufficient to determine test conditions (only nominal temperature & pho- toperiod given).				
	Metric 17:	Outcome Assessment Methodology	Medium	Dead flies were counted. $<5\%$ of flies escaped during the experiment & were excluded from the calculations of mortality.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes assessed consistently across groups.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables reported.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure reported.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	High	One-way ANOVA tables provided.				
	Metric 22:	Reporting of Data	High	Data reported for all outcomes.				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes reported.				
Additional Comments:	Because dos	e was not reported & cannot be back-calcu	lated with co	nfidence from the given information, this study is low-quality.				
Overall Qualit	ty Detern	nination	Low					

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Study Citation:	Chen, M. Y. junction, and	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.					
Duration: Exposure Route, Media. Path:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Invertebrate;	; Arthropods; Drosophila melanogaster; G	Canton Special	; Adult			
Health Outcome:	Ocular and S	Sensory					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5494836						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).			
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.			
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.			
	Metric 5:	Negative Control Response	High	Function of the retina measured by the electroretinogram: the biological response was expected to be adequate given the evidence in methodology provided by the authors.			
	Metric 6:	Randomized Allocation	Low	The study did not specifically state 'random allocation' or how organisms were allocated for the assessment.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% \text{ v/v}$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Finally, due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.			
	Metric 8:	Consistency of Exposure Administration	High	Function of the retina measured by the electroretinogram: There is no evidence showing that exposure administration of DEHP (0%, 0.1%, and 0.4%) for 20 days was not administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure of 20 days was adequate to evaluate whether DEHP expo- sure affects the function of the retina measured by the electroretinogram.			
		Con	tinued on nex	xt page			

Diethylhexyl Phthalate

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Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Terrestrial; Food/Diet; Dietary
Media, Path:	
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult
Health Outcome:	Ocular and Sensory
Chemical:	Di-ethylhexyl phthalate (DEHP)
HERO ID:	5494836

Domain	Metric	Rating	Comments
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Function of the retina measured by the electroretinogram: DEHP exposure groups: Ctrl (0%), 0.1%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).
Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.
Metric 15:	Number of Organisms and Replicates per Group	Low	Authors reported 8 or 9 flies per treatment group and at least 6 replicate electroretino- gram cycles for each fly, but they did not use treatment replicates per group.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (electroretinogram) addressed the intended out- come of interest (evaluation of the function of the retina).
Metric 18:	Consistency of Outcome Assessment	High	Details of the electroretinogram methodology to evaluate function of the retina were reported and assessed consistently across study groups.
Domain 6: Confounding / Variable Co	ontrol		
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.

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Study Citation: Duration: Exposure Route, Media Path:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; C	Canton Specia	l: Adult			
Health Outcome:	Ocular and Sensory	1	,			
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5494836					
Domain	Metric	Rating	Comments			
	Metric 21: Statistical Methods	High	Function of the retina measured by the electroretinogram: The difference between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for the post-hoc test.			
	Metric 22: Reporting of Data	High	Data was reported for all treatment group the text and Figure 3 and Supplementary Tables 5 and 6.			
	Metric 23: Explanation of Unexpected Outcomes	High	The authors reported standard error of the mean (Figure 3).			
Additional Comments:	Metric 23:Explanation of Unexpected OutcomesHighThe authors reported standard error of the mean (Figure 3).Summary notes:The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome:Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females					
Overall Qualit	ty Determination	High				

Study Citation:	Chen, M. Y. junction, and	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.						
Exposure Route,	Terrestrial; F	Ferrestrial; Food/Diet; Dietary						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Di-ethylhexy 5494836	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 5494836						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	Metric 1:	Test Substance Identity	High	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).				
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.				
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.				
Domain 2: Test Design								
C C	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.				
	Metric 5:	Negative Control Response	High	Courtship assay measured by copulation duration and latency: Based on the description and results by the authors the biological response of the control group (duration and latency of copulation) was adequate.				
	Metric 6:	Randomized Allocation	Medium	The authors indicated in Section 3.4 that Drosophila male and female virgins were ran- domly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP for 20 days.				
Domain 3: Exposure Cha	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% v/v$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.				
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consis- tently across study groups.				
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirm DEHP was dispersed after mixing, culture medium was examined by microscopy.				
Continued on next page								

Diethylhexyl Phthalate

		cont	inued from p	revious page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Uselth Outcomer	Chen, M. Y. junction, and Overall Dura Terrestrial; F Invertebrate;	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult							
Health Outcome: Chemical: HERO ID:	Di-ethylhexy 5494836	Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 5494836							
Domain		Metric	Rating	Comments					
	Metric 10:	Exposure Duration and Frequency	Medium	In the text (Section 3.4), the authors reported that Drosophila male and female virgins were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP for 20 days. However, Figure 7 indicates 10 days. Either 10 or 20 days exposure duration seemed adequate, but it is not clear what the actual duration was.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Courtship assay measured by copulation duration and latency: DEHP exposure groups were Ctrl (0%), 0.1%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).					
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.					
Domain 4: Test Organia	sm								
e.	Metric 13: Metric 14:	Test Organism Characteristics Acclimatization and Pretreatment Conditions	High High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays. Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.					
	Metric 15:	Number of Organisms and Replicates per Group	Low	In Section 2.6, the authors reported at least 60 pairs of flies per group. It is not clear what the exact number of pairs per treatment was or how the number of pairs differed across groups.					
Domain 5: Outcome As	ssessment								
	Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (copulation latency and duration) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on courtship behaviors).					
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treat- ment groups.					

Domain 6: Confounding / Variable Control

Diethylhexyl Phthalate

		contir	nued from p	revious page
Study Citation:	Chen, M. Y. junction, and	, Liu, H. P., Liu, C. H., Cheng, J., Chang, l courtship behaviors of Drosophila. Enviro	, M. S., Chi	ang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular lution 243(Pt B):1558-1567.
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days	
Exposure Route,	Terrestrial; F	food/Diet; Dietary		
Media, Path:				
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; Ca	nton Special	; Adult
Health Outcome:	Reproductive	e/Teratogenic		
Chemical:	Di-ethylhexy	l phthalate (DEHP)		
HERO ID:	5494836			
Domain		Metric	Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 7).
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figure 7 and Supplementary Table 9.
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.
Additional Comments:	Summary no	otes: The study evaluated the effects of E	EHP expos	ure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila

Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

Overall Quality Determination

High

Study Citation:	Liu, X., Li,	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend						
Duration	on AKT/FO	on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days						
Exposure Route,	Terrestrial; I	Terrestrial; Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Invertebrate:	; Arthropods; <i>Drosophila melanogaster</i> ; Ca	nton-S strain; Ad	lult				
Chemical:	Di-ethvlhexy	vl phthalate (DEHP)						
HERO ID:	11784619							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce	— — — — — — — — — —						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN as seen in Fig. 1.				
	Metric 2:	Test Substance Source	Low	they were analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.				
	Metric 5:	Negative Control Response	High	The control responses of the climbing tests were reported in Fig. 4 and were adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.				
Domain 3 [,] Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 100 flies placed in the test tube. There were three replicates per test group.				
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail. The test conditions such as temper- ature and relative humidity were not reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test for climbing behavior was reported to be 20 days. This appeared adequate for the outcome of interest.				
	Metric 11:	Number of Exposure Groups/	Low	There were only two exposure levels. This allowed for a comparison between a low and				
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	a high exposure concentration, but more exposure levels may have yielded more data. This exposure was via diet				
	110010 12.	Testing at or below bolubility Emilt	11/11	This exposure was the deci				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The Canton-S strain fruit flies were from Core Facility of Drosophila Resource and Technology at the CAS Center for Excellence in Molecular Cell Science. The flies were reported to be three days old at the start of the test.				
		Conti	nued on next pa	nge				

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Diethylhexyl Phthalate

		contin	ued from previ	ous page				
Study Citation:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/EOXO pathway. Taxicology In Vitro 95:105742							
Duration:	Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days							
Exposure Route.	Terrestrial: Food/Diet: Dietary							
Media Path								
Taxa Species Age	Invertebrate: Arthropods: Drosonhila melanogaster: Canton-S strain: Adult							
Health Outcome	Rehavioral							
Chemical:	Di-ethylbeyyl phthalate (DFHP)							
HFRO ID.								
	11/04019							
Domain		Metric	Rating	Comments				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the flies were acclimated prior to the start of the study.				
	Metric 15.	Conditions Number of Organisms and	Medium	It was reported that there were 100 flies per group with three replicates each for the				
	metrie 15.	Replicates per Group	Weardin	climbing test.				
				6				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–climbing behavior of the fruit flies after a chronic exposure.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Flies were placed in a glass crawling tube that measured 25cm after 20 days of feeding on the exposure diet. After slight oscillation of the tube to get the flies to the bottom, they all would begin to climb. A camera was used to take a photo after 4s of climbing. Average dis- tance climbed for each population tested was taken based off the distance climbed in the photo.				
Domain 6: Confounding	y / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	lysis						
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.				
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the climbing tests were re- ported in Fig. 4 and were adequate for the outcome of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.				

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Mortality						
Chemical: HERO ID:	Di-ethylhexyl phthalate (DEHP) 5494836						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		TT 1				
	Metric 1:	Test Substance Identity	Hıgh	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).			
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.			
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.			
	Metric 5:	Negative Control Response	High	The maximum survival time of the last control group fly was reported to be 77 days for males and 82 days for females. The survival rate in the control group was adequate as a typical, healthy and well-maintained outbred Drosophila population will have a median lifespan of approximately 70 days and maximum of approximately 90 days at 25 °C.			
	Metric 6:	Randomized Allocation	Medium	The authors reported in the results section 3.1 that Drosophila within three days after eclosion were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP.			
Domain 3: Exposure Characterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% \text{ v/v}$) was added to the mixture containing ethanol. To confirm DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.			
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consis- tently across study groups.			
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, and culture medium was examined by microscopy.			
Diethylhexyl Phthalate

	continued from previous page						
Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Terrestrial; Food/Diet; Dietary						
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult						
Health Outcome:	Mortality						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	5494836						

Domain		Metric	Rating	Comments
	Metric 10:	Exposure Duration and Frequency	High	The goal was to assess survival/mortality throughout the lifespan of Drosophila starting within three days after eclosion, while being continuously supplied with culture medium containing a series of concentrations of DEHP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Mortality assessment: DEHP exposure groups: Ctrl (0%), 0.05%, 0.1%, 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016). Observation: The text in Section 2.3 indicates that doses were 0, 0.05, 1, 2, 4%, but it is most likely a typo because in Figures 1 and 2 and everywhere else in the article, the authors reported 0, 0.05, 0.1, 0.2, and 0.4% DEHP.
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.
Domain 4 [.] Test Organis	m			
Domain in Test organis	Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 30-35 flies per replicate and seven replicates per treatment group.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.
	Metric 17:	Outcome Assessment Methodology	Medium	The authors reported assessing mortality when the vials with culture medium for fly maintenance were replaced twice a week and that during the replacement some flies ($<5\%$) escaped; flies that escaped were excluded from the calculation. It would have been most appropriate to determine and report how many flies per group were lost. While $<5\%$ might not be a high number, it is not clear if the authors made a general estimation that they were $<5\%$ or if they actually counted the number of flies that escaped.

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Diethylhexyl Phthalate

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Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.					
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Terrestrial; Fo	ood/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; Ca	nton Special	; Adult		
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5494836					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	Medium	The outcome (mortality) was assessed consistently across study groups, but it is not clear if the $<5\%$ of flies that escaped applies to all treatment groups equally.		
Domain 6: Confounding	/ Variable Con	trol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.		
Domain 7: Data Presenta	ation and Analy	/sis				
	Metric 21:	Statistical Methods	High	Mortality assessment: Authors used log-rank test and Gehan-Breslow-Wilcoxon test to determine the significance of the difference between survival curves.		
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figures 1A and 1B, and Supple- mentary Tables 1 and 2.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not report the SE or CI.		
Additional Comments:	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and					

and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

Overall Quality Determination High	
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Study Citation: Duration: Exposure Route,	Chen, M. Y. junction, and Overall Dura Terrestrial; F	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Behavioral Di-ethylhexy 5494836	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult Behavioral Di-ethylhexyl phthalate (DEHP) 5494836						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).				
	Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.				
	Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.				
	Metric 5:	Negative Control Response	High	The motor function of the control group measured by the climbing assay showed an expected result and that the control group climbed for efficiently that the two highest groups.				
	Metric 6:	Randomized Allocation	Medium	The authors reported in the results section 3.1 that Drosophila within 3 d after eclosion were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP.				
Domain 3: Exposure Ch	aracterization							
·	Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or $0.4\% \text{ v/v}$) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.				
	Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consis- tently across study groups.				
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.				
	Metric 10:	Exposure Duration and Frequency	High	The goal was to evaluate motor function measured by climbing assay throughout the lifespan of Drosophila following continuous exposure to DEHP.				
		Con	tinued on nex	xt page				

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	continued from previous page					
Study Citation:	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult					
Health Outcome:	Behavioral					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5494836					

Domain	Metric	Rating	Comments
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Motor function measured by climbing assay: DEHP exposure groups: Ctrl (0%), 0.05%, 0.1%, 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016). Observation: The text in Section 2.3 indicates that doses were 0, 0.05, 1, 2, 4%, but it is most likely a typo because in Figure 2 and everywhere else in the article, the authors reported 0, 0.05, 0.1, 0.2, and 0.4% DEHP.
Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in Drosophila studies ethanol has been com- monly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.
Oomain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Wild-type Drosophila melanogaster Canton Special (CS) wasused in all assays.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.
Metric 15:	Number of Organisms and Replicates per Group	Low	30-35 flies per replicate and 7 replicates per treatment group at the start of the exposure. However, as flies died, it is not clear how many organisms were used per group in the actual climbing assay to evaluate motor function.
Oomain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of Drosophila cultures.
Metric 17:	Outcome Assessment Methodology	Medium	To evaluate motor function/climbing activity, authors transferred the flies to a new vial and located them on the bottom of the vial on the culture medium, they agitated the vial and flies spontaneously started to climb upward along the vial. The authors reported that the whole process was video recorded and the number of flies that climbed over 5 cm within 15 s was counted. However, Figure 2 shows the climbing rate and the days (age) of the flies and the median and maximum climbing time. The data does not show the number of flies through time across treatment groups.

Diethylhexyl Phthalate

		contii	nued from p	revious page				
Study Citation:	Chen, M. Y. junction, and	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of Drosophila. Environmental Pollution 243(Pt B):1558-1567.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary						
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Drosophila melanogaster; Canton Special; Adult						
Health Outcome:	Behavioral		-					
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	5494836	• · · ·						
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome Assessment	Low	The outcome (motor function/climbing activity) was assessed consistently across study groups, but it is not clear if the $<5\%$ of flies that escaped applies to all treatment groups equally. Moreover, the actual number of flies included in the motor function/climbing activity is unclear.				
Domain 6: Confounding	g / Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Motor function/climbing activity assessment: Authors used log-rank test and Gehan- Breslow-Wilcoxon test to determine the significance of the difference in motor function.				
	Metric 22:	Reporting of Data	High	Data was reported for all treatment group the text and Figures 2A and 2B and Supple- mentary Tables 3 and 4.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not report SE or CI of climbing assay but they reported the 95% CI of calculated hazard ratio (Supplementary Tables 3 and 4).				

Additional Comments: Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (Drosophila melanogaster), (2) on neurological impacts such as climbing motor skills and electrophysyiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in Drosophila starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days.Notes on Drosophila lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

Overall Quality Determination

High

Study Citation:	Liu, X., Li, X on AKT/FOX	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.						
Duration: Exposure Route.	Overall Dura Terrestrial: F	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial: Food/Diet: Dietary						
Media, Path:	10110001100,1							
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; sir	t1 mutant; Adult					
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	11784619							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	The test substance was identified by CASPN as seen in Fig. 1				
	Metric 2:	Test Substance Source	Low	The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.				
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
2 oniani of 2nposaro on	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.				
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.				
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via diet.				

Domain 4: Test Organism

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 11784619 Table: 1 of 7

		contin	ued from previ	ous page				
Study Citation:	Liu, X., Li, on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.						
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Terrestrial; Food/Diet; Dietary							
Media, Path:		-						
Taxa, Species, Age:	Invertebrate	Arthropods; Drosophila melanogaster; sirt	1 mutant; Adult					
Health Outcome:	Mortality							
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	11784619							
Domain		Metric	Rating	Comments				
	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the flies were acclimated prior to the start of the study.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic.				
Domain 5: Outcome As	sessment							
Domain 5. Outcome ra	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.				
Domain 6: Confoundin	a / Variabla Ca	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	tation and Anal	vsis						
2 ciliuni /	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.				
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.				
Additional Comments:	This portion concentratio	of the evaluation is for the chronic exposu n of 10uM. They were exposed for the durat	are of the sirt1 i ion of their lives	mutant to DEHP. Three day old D. melanogaster were exposed to DEHP at one s and then lifespan was assessed.				

Overall Quality Determination

Study Citation: Duration: Exposure Route, Modio Dath:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster dep on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; akt mutant; Adult Mortality Di-ethylhexyl phthalate (DEHP) 11784619						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was identified by CASRN as seen in Fig. 1. The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2. Test Design							
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 3. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						
<u>G</u> ara	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.			
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HERO ID: 11784619 Table: 2 of 7

		contin	ued from previ	ous page			
Study Citation: Duration:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; akt	mutant; Adult				
Health Outcome: Chemical:	Mortality Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	11784619	() productice (D 2111)					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.			
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion concentratio	of the evaluation is for the chronic expose n of 10uM. They were exposed for the durat	ure of the akt n ion of their lives	nutant to DEHP. Three day old D. melanogaster were exposed to DEHP at one s and then lifespan was assessed.			

Overall Quality Determination

Study Citation: Duration: Exposure Route, Madia, Path:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Di-ethylhexy 11784619	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; atg13 mutant; Adult Mortality Di-ethylhexyl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce	neure	ituting	Commons			
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was identified by CASRN as seen in Fig. 1. The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Test Design	Metric 4 [.]	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the			
	metric ii	reguire contons	mgn	exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.			
Continued on next page							

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Environmental Hazard Evaluation

		contin	ued from previ	ous page		
Study Citation:	Liu, X., Li, on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	days			
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; atgl	3 mutant; Adul	t		
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	11784619					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic.		
Domain 5: Outcome A	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.		
Domain 6: Confoundin	og / Variable Co	ntrol				
Domain 0. Comoundin	Metric 19.	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	methe 19.	Design and Procedures	Low	conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presen	ntation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.		
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion concentratio	of the evaluation is for the chronic exposu n of 10uM. They were exposed for the durat	re of the atg13 ion of their lives	mutant to DEHP. Three day old D. melanogaster were exposed to DEHP at one and then lifespan was assessed.		

Overall Quality Determination

Study Citation: Duration: Exposure Route,	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate: Mortality Di-ethylhexy 11784619	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; p53 mutant; Adult Mortality Di-ethylhexyl phthalate (DEHP) 11784619					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was identified by CASRN as seen in Fig. 1. The DEHP and DINP were both reported to be from Sigma, but it was not reported if			
	Metric 3:	Test Substance Purity	High	they were analytically verified by the performing laboratory. Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Test Design	Matria 4	Negative Controls	TT:-1				
	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 11784619 Table: 4 of 7

		contin	ued from previ	ious page		
Study Citation:	Liu, X., Li, on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.				
Duration:	Overall Dur	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; p53	mutant; Adult			
Health Outcome:	Mortality					
Chemical:	Di-ethylhex	yl phthalate (DEHP)				
HERO ID:	11784619					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic.		
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.		
Domain 6: Confoundin	g / Variable Co	ntrol				
Domain of Comountain	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presen	tation and Anal	lvsis				
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.		
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion concentration	n of the evaluation is for the chronic exposu n of 10uM. They were exposed for the durat	are of the p53 r ion of their lives	nutant to DEHP. Three day old D. melanogaster were exposed to DEHP at one s and then lifespan was assessed.		

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media Path:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome:	Invertebrate: Mortality	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; tor mutant; Adult Mortality					
HERO ID:	11784619	yi phthalate (DEHP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	Trat Calendary of Identities	II: -1-				
	Metric 1: Metric 2:	Test Substance Source	Low	The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						
C	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.			
Continued on next page							

HERO ID: 11784619 Table: 5 of 7

		contin	ued from previ	ous page			
Study Citation:	Liu, X., Li, I on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	days				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary	2				
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; tor 1	mutant; Adult				
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	11784619						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Medium	It was reported that there were 80-100 flies per group with three replicates each for the			
		Replicates per Group		chronic.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.			
Domain 6: Confounding	🔊 / Variable Co	ntrol					
2 oniani or contounany	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.			
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion concentratio	of the evaluation is for the chronic expose n of 10uM. They were exposed for the durat	ure of the tor m ion of their lives	nutant to DEHP. Three-day old D. melanogaster were exposed to DEHP at one s and then lifespan was assessed.			

Overall Quality Determination

Study Citation:	Liu, X., Li, Z on AKT/FOZ	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.						
Duration: Exposure Route, Media Path:	Overall Dura Terrestrial; F	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Canton-S strain; Adult							
Health Outcome:	Mortality	Mortality						
Chemical:	Di-ethylhexy	-ethylhexyl phthalate (DEHP) 784619						
HERO ID:	11/84619	1784619						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	Metric 1:	Test Substance Identity	High	The test substance was identified by $CASPN$ as seen in Fig. 1				
	Metric 2:	Test Substance Source	Low	The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.				
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the				
	Wether 4.	Regative controls	Ingn	exposure.				
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Table 3 and Fig. 3 and were adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.				
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.				
	Metric 11:	Number of Exposure Groups/	Low	There were only two exposure levels. This allowed for a comparison between a low and				
	Matric 12:	Spacing of Exposure Levels	N/A	a high exposure concentration, but more exposure levels may have yielded more data.				
		resting at or below Solubility Lillit	1N/A	This exposule was via ulet.				
Domain 4: Test Organis	m							
·	Metric 13:	Test Organism Characteristics	High	The Canton-S strain fruit flies were from Core Facility of Drosophila Resource and Technology at the CAS Center for Excellence in Molecular Cell Science. The flies were reported to be three days old at the start of the test.				
Continued on next page								

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		contin	ued from previ	ous page		
Study Citation:	Liu, X., Li, on AKT/FO	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742.				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; I	Terrestrial; Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; Can	ton-S strain; Ad	ult		
Health Outcome:	Mortality					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	11784619					
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the flies were acclimated prior to the start of the study.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic test.		
Domain 5: Outcome As	sessment					
Domain 5. Outcome As	Metric 16	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.		
Domain 6 [,] Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Ana	lysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.		
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 3 and Table 3 and were adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion of the evaluation is for the chronic exposure to DEHP. Three-day old D. melanogaster were exposed to DEHP at two concentrations of 1uM and 10uM. They were exposed for the duration of their lives and then lifespan was assessed. There were also different diets, a normal diet and a high fat diet					
Overall Qualit	ty Deterr	nination	Medium			

Study Citation: Duration: Exposure Route,	 Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila m on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary 						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate: Mortality Di-ethylhexy 11784619	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; foxo mutant; Adult Mortality Di-ethylhexyl phthalate (DEHP) 11784619					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was identified by CASRN as seen in Fig. 1. The DEHP and DINP were both reported to be from Sigma, but it was not reported if they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Punty	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the			
	Metric 5:	Negative Control Response	High	The control responses of the chronic tests were reported in Fig. 5 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. There were 80-100flies per test group, but it is unclear how many flies there were per tube. There were three replicates per test group.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the chronic test was for the lifespan of the fruit flies. This varied based on the treatment. This was adequate for assessing the outcome of interest.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This exposure was via diet.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The mutant flies were from Tsinghua University. It was reported that three-day old flies were used in the lifespan studies.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the flies were acclimated prior to the start of the study.			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 11784619 Table: 7 of 7

		contin	ued from previ	ous page		
Study Citation:	Liu, X., Li, on AKT/FO	X., Liu, Y., Wu, W. D., Liu, X. M. (2024). I XO pathway. Toxicology In Vitro 95:105742	DEHP and DIN	P accelerate aging effects in male and female of Drosophila melanogaster depend		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Drosophila melanogaster; foxo	o mutant; Adult			
Health Outcome:	Mortality					
Chemical:	Di-ethylhex	yl phthalate (DEHP)				
HERO ID:	11784619					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 80-100 flies per group with three replicates each for the chronic test.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lifespan for the duration of the exposure.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. Fruit flies were exposed for the duration of their life. Flies were counted every day where deaths were tallied. They were moved to new chambers with the same diet/treatment every week until all flies had died.		
Domain 6: Confounding	a / Variable Co	ntrol				
Domain 0. Comountain	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7: Data Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.		
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the chronic tests were re- ported in Fig. 5 and were adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion concentratio	of the evaluation is for the chronic exposu n of 10uM. They were exposed for the durat	re of the foxo it ion of their lives	mutant to DEHP. Three day old D. melanogaster were exposed to DEHP at one s and then lifespan was assessed.		

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path:	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/FOXO pathway. Toxicology In Vitro 95:105742. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Di-ethylhexy 11784619	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; No strain reported; Adult Mechanistic-Cell signaling/function Di-ethylhexyl phthalate (DEHP) 11784619					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN as seen in Fig. 1.			
	Metric 2:	Test Substance Source	Low	The DEHP and DINP were both reported to be from Sigma, but it was not reported if			
			TT: 1	they were analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Both the DEHP and DINP had a purity of 99.5%.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a DMSO control that was run concurrently with the exposure.			
	Metric 5:	Negative Control Response	High	The control responses of the gene expression tests were reported in Fig. 6 and were adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the flies were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure medium was prepared by adding DINP or DEHP to the food medium used for the fruit flies. DMSO was used to dilute the test compounds prior to adding them to the medium. The medium was autoclaved prior to the addition of the DEHP or DINP. Information was lacking regarding the test system, however. It is unclear what the dimensions of the test chamber were other than that they were tubes. The number of flies used in each exposure was unclear.			
	Metric 8:	Consistency of Exposure Administration	Low	The test chambers were not described in great detail, nor was it specified how many flies were in each test chamber. The test conditions such as temperature and relative humidity were not reported.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in this study.			
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	The exposure duration for this portion of the test was not reported other than to say it was "chronic." There were different exposure periods reported for other chronic expo- sures discussed in this paper, and it was unclear if one of those durations was used for this portion of the test or if a completely different duration was used.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one test concentration for this portion of the exposure.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This exposure was via diet.			

Domain 4: Test Organism

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Diethylhexyl Phthalate

		cont	tinued from previous	s page			
Study Citation:	Liu, X., Li, Z	Liu, X., Li, X., Liu, Y., Wu, W. D., Liu, X. M. (2024). DEHP and DINP accelerate aging effects in male and female of Drosophila melanogaster depend on AKT/EOXO pathway. Toxicology In Vitro 95:105742					
Duration:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported					
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	; Arthropods; Drosophila melanogaster; No stra	ain reported; Adult				
Health Outcome:	Mechanistic	-Cell signaling/function					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	11784619						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	Low	The strain of the flies for this portion of the test was not reported, nor was the age.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the flies were acclimated prior to the start of the study.			
	Metric 15.	Conditions Number of Organisms and	Low	The number of flies per exposure was not reported for this portion of the test. It was			
	Methe 15.	Replicates per Group	Low	reported that the experiment was replicated three times.			
		Tophewer per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Little details were provided on the culturing conditions and the test conditions.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-gene expression.			
	Metric 18:	Consistency of Outcome	High	Outcomes appeared to be assessed consistently across study groups. Fly samples were			
		Assessment		assessed via RT-qPCR. A kit was used to isolate the RNA.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	veie					
Domain 7. Data i lesen	Metric 21:	Statistical Methods	High	Statistical analysis was described in section 2.6 and was adequate for the outcomes of interest.			
	Metric 22:	Reporting of Data	High	The responses for the controls and the exposure groups for the gene expression tests were reported in Fig. 6 and were adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion not reported	of the evaluation is for gene expression after ex how long the exposure was other than to say it	posure to DEHP. D. r was a "chronic" expo	nelanogaster were exposed to DEHP at one concentration of 10uM. It was osure, thus the unacceptable rating.			

Overall Quality Determination

Uninformative

Study Citation:	Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm Eisenia					
Duration: Exposure Route, Media, Path:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route					
Taxa, Species, Age:	Invertebrate;	; Arthropods; Eisenia fetida; Savigny; Adult	t			
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	3625226					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce	— • • • •				
	Metric 1:	Test Substance Identity	High	The test substance was listed by name and CAS number.		
	Metric 2:	Test Substance Source	Hıgh	"The chemicals were purchased from the Aldrich Chemical Co., Milwaukee, WI, East- man Kodak Co., Rochester, NY, and Fisher Scientific Co., Fairlawn, NJ."		
	Metric 3:	Test Substance Purity	High	A minimum purity of 98% was stated.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Controls were utilized.		
	Metric 5:	Negative Control Response	Low	A negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was unclear whether DEHP was dissolved in a solvent prior to addition to filter paper.		
	Metric 8:	Consistency of Exposure Administration	Medium	It is unclear if a solvent was utilized and if the same amount of solvent was used across the different treatment groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	This study utilized a 48-hr contact exposure duration to establish an LC50 value.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Exposure concentrations were not stated. At least five concentrations were utilized for the definitive test (exact number of exposure groups not specified). It is unclear whether one of these concentrations was a control (blank)		
	Metric 12:	Testing at or Below Solubility Limit	Low	The test substance was applied to filter paper using water or other solvent. It is unclear which was used and if concentrations exceeded solubility limit.		
Domain 4: Test Organis	m					
-	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not stated.		
	Metric 14:	Acclimatization and Pretreatment	Low	An acclimation process/procedure was not reported.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 10 replicate worms per test concentration (1 worm per vial).		
Domain 5: Outcome Assessment Matrin 16: Adaguagy of Tast Conditions Madium It is used as the destant of the destant of the second state of the sec						
Continued on next page						

Diethylhexyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Neuhauser, l fetida. Journ	Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm Eisenia fetida. Journal of Environmental Quality 14(3):383-388.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Eisenia fetida; Savigny; Adult					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	3625226						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed using a "gentle mechanical touch".			
	Metric 18:	Consistency of Outcome Assessment	High	Mortality was assessed in study groups after the 48-hour exposure duration.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Attrition in each treatment group was not reported; however there was no information to suggest differences in attrition among the study groups unrelated to exposure.			
Domain 7: Data Presen	tation and Anal	lysis					
	Metric 21:	Statistical Methods	High	"The LC50 value for each chemical tested was calculated using the method of Litchfield and Wilcoxon (1949)."			
	Metric 22:	Reporting of Data	Low	LC50 values with CI were reported in Table 1, but data for each individual treatment group was not shown.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Confidence intervals were reported.			
Additional Comments:	This form is	for the contact test. DEHP and DBP were n	ot selected for tl	ne artificial soil test.			
Overall Quali	ty Deterr	nination	Medium				

Page 851 of 958

Study Citation:	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia					
Duration: Exposure Route, Media. Path:	Thetaria. Er Overall Dura Terrestrial; S	ation: 4 - 10 days; Exposure Duration: 4 - 16 Soil; Not determined by study authors (i.e., c	5):1085-1091. 0 days chemical of inter	est in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Arthropods; Folsomia fimetaria; Juvenile					
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	789786					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce	T (C) ()	Ŧ			
	Metric 1:	Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.		
	Metric 2:	Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A negative control was used (acetone-water).		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Damain 2. Enna ann Ch						
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	Experiments were conducted in small multidish chamber vessels with spiked soil. The exposure duration for this experiment was one week, and the experimental results for the degradation test (Figure 1) show that degradation after one week was greater than 20%. This may have an impact on results, though not as great as the impact for the longer experiments reported.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.		
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at begin- ning of experiment).		
	Metric 11:	Number of Exposure Groups/	High	"Test concentrations were DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight."		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	"Ten juveniles (0-1 d old) were added to each of two replicated microcosms."		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no reported acclimatization, but there was no evidence to suggest results were impacted.		
		Conti	nued on next pa	nge		

Diethylhexyl Phthalate

		continu	ued from previo	ous page			
Study Citation:	Jensen, J., L fimetaria. Er	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e., cl	hemical of intere	est in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Folsomia fimetaria; Juvenile					
Health Outcome:	Mortality						
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	789786						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Medium	There were 10 juveniles per concentration, and two replications.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing, environmental conditions, food, water, nutrients and/or biomass loading was not sufficiently reported to evaluate if adequate and whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	High	Juveniles were assessed for survival after one week of exposure.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment	_				
Domain 6: Confounding	/ Variable Co	ntrol					
Domain of Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures	2011	conditions or other non-treatment-related factors across study groups.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	veis					
Domain 7. Dua Present	Metric 21:	Statistical Methods	High	Details of the statistical methods used were reported in the methods section, and they were appropriate for the study.			
	Metric 22:	Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualita- tively. "Juvenile mortality was similar when exposed in multidishes with a hard soil surface and in microcosms with loose soil (data not shown)."			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						

Overall Quality Determination

Study Citation:	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia				
Duration: Exposure Route, Media. Path:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate: Reproductiv Di-ethylhexy 789786	; Arthropods; <i>Folsomia fimetaria</i> ; Adult e/Teratogenic yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.	
	Metric 2:	Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5:	Negative Control Response	High	Biological responses in the control were normal.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Experiments were conducted in microcosm vessels with spiked soil. Because of the length of the exposure (22 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil. Concentrations of the test substance were not measured during the study.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at begin- ning of experiment).	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations used were DEHP at 0, 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg dry weight."	
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.	
Domain 4: Test Organia	m				
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	Male and female adult collembolans were used	
	Metric 14:	Acclimatization and Pretreatment	Low	There was no reported acclimatization, but there was no evidence to suggest results were impacted	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten males and ten females per microcosm, and four replicates per concentra- tion.	
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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

Diethylhexyl Phthalate

HERO ID: 789786 Table: 1 of 2

		contir	nued from previo	bus page		
Study Citation:	Jensen, J., L fimetaria. Er	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	- 21 days			
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e., c	chemical of intere	est in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Folsomia fimetaria; Adult				
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	789786					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and test conditions were acceptable. Experiments were conducted at a constant temperature (20C), with a 12:12 h light:dark regime. Animals were fed dried baker's yeast (15 mg dry weight) at day 0 and day 14.		
	Metric 17:	Outcome Assessment Methodology	Medium	Reproduction was assessed by counting surviving juveniles at the end of the study using digital image processing. However, juvenile mortality during the test period was not assessed, and was only counted at the end of the experiment.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups (at the end of the experiment)		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	lvsis				
	Metric 21:	Statistical Methods	High	Details of statistical methods were reported in the methods and were appropriate for the study.		
	Metric 22:	Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualita- tively or quantitatively. EC10/LC10/EC50/LC50 were reported for all endpoints.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Ouali	tv Deterr	nination	Medium			

Study Citation:	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.				
Duration: Exposure Route,	Overall Dura Terrestrial; S	ation: 11 - 21 days; Exposure Duration: 11 - Soil; Not determined by study authors (i.e., c	21 days chemical of inter	est in exposure water, but unable to determine exact uptake route)	
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate: Mortality Di-ethylhexy 789786	Arthropods; <i>Folsomia fimetaria</i> ; Adult yl phthalate (DEHP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	Test substance identified only by nomenclature. No other information (CASRN, struc- ture etc.) was provided.	
	Metric 2:	Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5:	Negative Control Response	High	Biological responses in control were normal.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3. Exposure Ch	aracterization				
Domain D. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	Experiments were conducted in microcosm vessels with spiked soil. Because of the length of the exposure (22 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at begin- ning of experiment).	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations used were DEHP at 0, 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg dry weight."	
	Metric 12:	Testing at or Below Solubility Limit	N/A	Spiked soil exposure.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	Male & female adult collembolans were used.	
	Metric 14:	Acclimatization and Pretreatment	Low	No reported acclimatization, but no evidence to suggest results impacted.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 male & 10 female per microcosm, 4 replicates per concentration.	
Domain 5: Outcome As	sessment				

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Diethylhexyl Phthalate

	continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Jensen, J., L fimetaria. Er Overall Dura Terrestrial; S Invertebrate; Mortality	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	789786					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Organism housing & conditions acceptable. "Experimentswere conducted at constant temperature (20oC), with a12:12 h light: dark regime. Animals were fed dried baker's yeast (15 mg dry weight) at day 0 and day 14".		
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed using digital image processing.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups (at the end of the experiment).		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Details of statistical methods reported in methods & are appropriate for the study.		
	Metric 22:	Reporting of Data	High	No effects were found at any concentration. EC10/LC10/EC50/LC50 are reported for all endpoints.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Concentratic reported. Th	Concentration of DEHP in soil was not measured during or at the end of the experiment. Environmental conditions (moisture content, pH, etc.) were not reported. There was no effect of DEHP on adult survival at the concentrations tested.				

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration:	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial: Soil: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route).				
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e., cr	iemical of inter	rest in exposure water, but unable to determine exact uptake route)	
Media, Path:	T (1)				
Taxa, Species, Age:	Invertebrate	; Arthropods; Folsomia fimetaria; Juvenile			
Health Outcome:	Mortality	al al-th-late (DEUD)			
UEDO ID.	Di-ethylnex	yi phthalate (DEHP)			
HERO ID:	/89/86				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.	
	Metric 2:	Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Experiments were conducted in small multidish chamber vessels with spiked soil. Be- cause of the length of the exposure (60 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil. Concentrations of the test substance were not measured during the study.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Measurement of Test Substance	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at begin- ning of experiment).	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations were DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight."	
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	A single 0 to 1-d old juvenile springtail was added to each chamber.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no reported acclimatization, but also no evidence to suggest results were impacted.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 20 organisms per exposure concentration, and no replicates.	
Continued on next page					

		conti	nued from previo	us page		
Study Citation: Duration: Exposure Route, Media Path:	Jensen, J., L fimetaria. Er Overall Dura Terrestrial; S	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Invertebrate; Arthropods; Folsomia fimetaria; Juvenile					
Health Outcome:	Mortality					
HERO ID:	789786	(DEIII)				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and test conditions were acceptable. "Experiments were run at con- stant temperature (20oC), with a 12:12 h light:dark regime."		
	Metric 17:	Outcome Assessment Methodology	Medium	Juveniles were assessed twice a week for six weeks.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups (twice a week for six weeks).		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions (pH, moisture content, etc.) or other non-treatment-related factors across study groups.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Details of the statistical methods used were reported in the methods, and were appropri- ate for the study.		
	Metric 22:	Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualita- tively or quantitatively. EC10/LC10/EC50/LC50 are reported for all endpoints.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quali	ty Detern	nination	Medium			

Study Citation:	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia					
D	fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.					
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: > 21 days Terrestrich: Soil: Not determined by study authors (i.e., shomical of interest in exposure water, but unable to determine exact untake route)					
Exposure Route, Modio Doth	refrestriar, son, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Town Spacing Ages						
Taxa, Species, Age:	Development	Artinopous, <i>Foisomia jimetaria</i> , Juvenne				
Chamiagh	Developmen	II/GIOWIN				
	780786	yi phinalale (DEHP)				
	/89/80					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	Matria 1.	Test Substance Identity	Law	Test substance identified only have superior No. they information (CACDN) stress		
	Metric 1:	Test Substance Identity	Low	ture etc.) was provided.		
	Metric 2:	Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design	N		TT: 1			
	Metric 4:	Negative Controls	High	A negative control was used (acetone-water).		
	Metric 5:	Negative Control Response	High	Biological responses in control were normal.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3 [,] Exposure Ch	aracterization					
Domain 5. Exposure of	Metric 7:	Experimental System/Test Media	Medium	Experiments were conducted in small multidish chamber vessels with spiked soil. Be-		
		Preparation		cause of the length of the exposure (60 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	DEHP concentrations were not measured throughout the study, and may be expected to		
	Matria 10.	Concentration	II: -h	degrade in soil. This may have a substantial impact on results.		
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at begin- ning of experiment).		
	Metric 11:	Number of Exposure Groups/	High	"Test concentrations were DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight"		
	Matria 12.	Spacing of Exposure Levels	NT/A	Spiled soil appropria		
	Weute 12.	Testing at of Below Solubility Linit	IN/A	Spiked son exposure.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	A single 0 to 1-d old juvenile springtail was added to each chamber.		
	Metric 14:	Acclimatization and Pretreatment	Low	No reported acclimatization, but no evidence to suggest results impacted.		
	16 . 1 . 15	Conditions	T			
	Metric 15:	Number of Organisms and	Low	20 organisms per exposure concentration, no replicates.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing & conditions acceptable.		
		Conti	nued on next na	ge		
Commune on next page						

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		conti	nued from previo	bus page			
Study Citation: Duration:	Jensen, J., L fimetaria. Er Overall Dura	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091. Overall Duration: 4 - 10 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:							
Taxa, Species, Age: Health Outcome:	Developmen	Arthropods; <i>Foisomia jimetaria</i> ; Juvenile					
Chemical	Di-ethylbey	/l phthalate (DEHP)					
HERO ID:	789786						
Domain	Metric Rating Comments						
	Metric 17:	Outcome Assessment Methodology	Medium	"During the first three weeks, covering an entire F. fimetarialife cycle, exuviae of grow- ing juveniles were recorded everysecond day and removed if present.""Growth of the animals was determined manually at the screen by measuring the length from the poste- rior end of the abdomen tothe anterior end of the head."			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	y / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Details of statistical methods reported in methods & are appropriate for the study.			
	Metric 22:	Reporting of Data	High	No effects were found at any concentration. EC10/LC10/EC50/LC50 are reported for all endpoints.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most						
Duration: Exposure Route, Modia, Pathy	Widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; N/A (e.g., injection); Dietary						
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult						
Health Outcome:	Behavioral						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2345940						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	Metric 1.	Test Substance Identity	High	The chemical was identified by name and CAS number			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical was reported as pure.			
Domain 2: Test Design							
6	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Characterization							
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	Only one test concentration was used (food choice test).			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via food choice, so solubility limit is not applicable.			
Domain 4: Test Organism							
	Metric 13:	Test Organism Characteristics	Medium	There were minor uncertainties about the characteristics of the test organisms.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and	Low	The number of replicates were not reported.			
		Replicates per Group					
Domain 5: Outcome Assessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health, although there were few details provided.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Terrestrial; N/A (e.g., injection); Dietary Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Lasius niger; Adult **Health Outcome:** Behavioral Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 2345940 Domain Metric Rating Comments Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low The study did not provide enough information to allow a comparison of environmental conditions. Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical analysis was performed and described adequately. Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Cuvillier-Ho widespread p Overall Dura Terrestrial; N	 ²uvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ²Uvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ²Uvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ³Uvillier-Hot, V., Salin, K., Oregan, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ³Uvillier-Hot, V., Salin, K., Oregan, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ⁴Uvillier-Hot, V., Salin, K., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. ⁴Uvillier-Hot, V., Salin, K., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most videspread phthalate on a terrestrial species of the most videspread phthalate on a terrestrial species of the most videspread phthalate on a terrestrial species of the most videspread phthalate on a terrestrial species of terrestrial species of terrestrial species of terr					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics Di-ethylhexyl phthalate (DEHP) 2345940						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS number.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical reported as pure.			
Domain 2. Test Design							
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.			
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in			
		Preparation	C	adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration used.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via topical application.			
Domain 4: Test Organis	m						
c	Metric 13:	Test Organism Characteristics	Medium	Minor uncertainties about the characteristics of test organisms.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and	Low	The use of replicates was not reported.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health although there were few details.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest with few details.			
Continued on next page							

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Exposure Route, Terrestrial; N/A (e.g., injection); Dermal (topical application) Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Lasius niger; Adult **Health Outcome:** Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 2345940 Domain Metric Rating Comments Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Confounding Variables in Test Metric 19: Low The study did not provide enough information to allow a comparison of environmental Design and Procedures conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups in animal attrition. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods N/A Statistical analysis was not performed, results were assessed as levels of expression. High Metric 22: Reporting of Data Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes Low There was no clear response. Additional Comments: gene expression

Overall Quality Determination

Medium

Study Citation:	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110.						
Duration: Exposure Route,	Overall Dura Terrestrial; N	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; N/A (e.g., injection); Dermal (topical application)					
Media, Path: Taxa Species Age	Invertebrate	Arthropods: Lasius niger: Adult					
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2345940						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS number.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical reported as pure.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.			
Domain 3: Exposure Ch	Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration used.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via topical application.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Minor uncertainties about the characteristics of test organisms.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The use of replicates was not reported.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health although there were few details.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Cuvillier-Ho	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most				
	widespread p	widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	s (0-96h)		
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Dermal (topical applic	ation)			
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Lasius niger; Adult				
Health Outcome:	Mechanistic	Oxidative stress (including redox biology)				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2345940					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Matria 20.	Design and Procedures Outcomes Unrelated to Exposure	Madium	Conditions.		
	Wieurie 20.	Outcomes Omerated to Exposure	Wedfulli	attrition.		
Domain 7: Data Present	ation and Anal	veis				
Domain 7. Data Present	Metric 21:	Statistical Methods	High	Statistical analysis was performed and described adequately		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	Low	There was no clear response.		
Additional Comments:	oxidative dat	mage (Lipid peroxidation, TBARS)				
Overall Quality Determination High						

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Study Citation:	Lenoir, A., 7	Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. Environmental					
Duration: Exposure Route, Media. Path:	Science and Overall Dura Terrestrial; N	Science and Pollution Research 21(23):13446-13451. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; N/A (e.g., injection); Dermal (topical application)					
Taxa, Species, Age:	Invertebrate; Arthropods; Lasius niger; Adult						
Health Outcome:	ADME (biotransformation)						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2347468	2347468					
Domain	Metric Rating Comments						
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	The chemical was 99% pure.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Characterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	Medium	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were measured after administration and presented as time 0 within Figure 1 on page 4/6.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	N/A	Only one dose was used.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The test chemical was solubilized in methanol prior to topical application.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome Ass	Metric 16:	Adequacy of Test Conditions	Low	Minor uncertainties were identified regarding environmental conditions of the test sys- tem due to the lack of details provided.			
Continued on next page							

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Diethylhexyl Phthalate

... continued from previous page **Study Citation:** Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. Environmental Science and Pollution Research 21(23):13446-13451. Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; N/A (e.g., injection); Dermal (topical application) **Exposure Route**, Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Lasius niger; Adult **Health Outcome:** ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) **HERO ID:** 2347468 Comments Domain Metric Rating Metric 17: Outcome Assessment Methodology Medium The outcome assessment methodology reported the intended outcomes of interest with some uncertainty. Metric 18: Consistency of Outcome Medium There was incomplete reporting of minor details of outcome assessment protocol execution. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among the study groups in environmental conditions. **Design and Procedures** Metric 20: Outcomes Unrelated to Exposure Medium There were no differences among groups, but there were few details to confirm that. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Low Statistical analysis was performed but not described adequately. Metric 22: High Reporting of Data Data for exposure-related findings were presented for each treatment and control group. Metric 23: Explanation of Unexpected Outcomes Medium Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most					
Duration: Exposure Route, Media Path	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; N/A (e.g., injection); Dermal (topical application)					
Taxa, Species, Age:	Invertebrate:	Arthropods: Lasius niger: Adult				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	/l phthalate (DEHP)				
HERO ID:	2345940	-				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS number.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	The chemical was reported as pure.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was used.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via topical application.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	There were minor uncertainties about the characteristics of test organisms.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.		
	Metric 15:	Conditions Number of Organisms and	Low	The use of replicates was not reported.		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health, although there were few details provided.		
		Cont	inued on nex	xt page		
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Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Cuvillier-Ho widespread t	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant Lasius niger. Environmental Research 131:104-110.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 2	l days				
Exposure Route,	Terrestrial; N	J/A (e.g., injection); Dermal (topical applied	cation)				
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Lasius niger; Adult						
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2345940						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding / Variable Control							
c c	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal			
				attrition.			
Damain 7. Data Draamt							
Domain /: Data Present	Matria 21.	ysis Statistical Matheda	Iliah				
	Metric 21:	Statistical Methods	пign Ui-h	Statistical analysis was performed and described adequately.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	I nere were no unexpected outcomes.			
Additional Comments:	This evaluati	ion is for egg laying rate.					
Overall Quality Determination		High					

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., S. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth S littoralis. Chemosphere 215:725-738. Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Exposure Route, Media, Path: Terrestrial; Food/Diet; Dietary Media, Path: Invertebrate; Arthropods; Spodoptera littoralis; Larvae Health Outcome: ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5494137 Domain Metric Metric 1: Test Substance Identity Low Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. structure, or other chemical descriptors were not reported. Metric 2: Test Substance Identity Low Direthical analytically. Domain 1: Test Design Metric 3: Test Substance Purity Low Purity was not reported. Domain 2: Test Design Metric 4: Negative Control Response High Solvent control (0.5% ethanol) was used. Domain 2: Test Design Metric 5: Negative Control Response High Solvent control (0.5% ethanol) was used.	iussat. D.						
Exposure Route, Media, Path: Terrestrial; Food/Diet; Dietary Media, Path: Invertebrate; Arthropods; Spodoptera littoralis; Larvae Health Outcome: ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5494137 Domain 1: Test Substance Metric 1: Test Substance Identity Low Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. structure, or other chemical descriptors were not reported. Metric 2: Test Substance Identity Low Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. structure, or other chemical substance was obtained from PESTANAL® 36735, Sigma, Fra authors did not verified analytically. Metric 3: Test Substance Purity Low Purity was not reported. Domain 2: Test Design Metric 4: Negative Controls Metric 5: High Solvent control (0.5% ethanol) was used. Metric 5: Negative Control Response Medium Control response was reported. The authors noted that DEHP is present in th ronment and food at low background concentrations - the diet of controls con runnes of 202 + 105 me of DEUD mere of 202 + 105 me of DEUD mere of	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days						
Taxa, Species, Age: Invertebrate; Arthropods; Spodoptera littoralis; Larvae Health Outcome: ADME (biotransformation) Chemical: Di-ethylhexyl phthalate (DEHP) HERO ID: 5494137 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity Low Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. structure, or other chemical descriptors were not reported. Metric 2: Test Substance Source Low Dicemical substance was obtained from PESTANAL® 36735, Sigma, Fraauthors did not verified analytically. Domain 2: Test Design Metric 4: Negative Controls High Solvent control (0.5% ethanol) was used. Metric 5: Negative Control Response High Solvent control (0.5% ethanol) was used. Control response was reported. The authors noted that DEHP is present in the ronment and food at low background concentrations - the diet of controls concentrat	Terrestrial; Food/Diet; Dietary						
Internet of the second of t	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae ADME (biotransformation)						
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Metric 5: Negative Control Response Medium Control response was reported. The authors noted that DEHP is present in the ronment and food at low background concentrations - the diet of controls concurrence of 202 + 125 ng of DEHP page g							
average of 392 ± 123 lig of DELTP per g.	envi- tained an						
Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups.							
Domain 3: Exposure Characterization							
Metric 7: Experimental System/Test Media Low The study provided limited details about how the doses in the diet were obtained by the doses in the do	ned, but a						
Preparation description was provided as to how doses were measured/validated.	,						
Metric 8: Consistency of Exposure Medium Unclear how the diet was spiked with the test material.							
Metric 9: Measurement of Test Substance High Doses in the diet were confirmed analytically (GC-MS) - methods were desc	ibed in 2.2						
Metric 10: Exposure Duration and Frequency Low The exposure duration was from the 3rd instar to pupation. The authors repo measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old) unclear how many days the study duration was.	ted taking but it is						
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels High Spacing of Exposure Levels High Spacing of Exposure Levels High High Spacing of Exposure Levels High Spacing of Exposure	10 ng; 100 5 mg and d in food- asurement contami-						
Metric 12: Testing at or Below Solubility Limit N/A Exposure was via diet.							

Domain 4: Test Organism

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Diethylhexyl Phthalate

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae						
Health Outcome:	ADME (bio	ADME (biotransformation)					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5494137						
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The authors reported in the figure caption (Figures 1 and2) that for analysis of DEHP in food, they used $n = 6$ to 11 for each condition; for analysis of DEHP in organisms, they used $n = 3 - 4$ biological replicates for each condition corresponding to a pool of 3 - 10 individuals).			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not suffi- ciently reported to evaluate whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	High	The authors measured concentrations of DEHP in various larval stages and confirmed concentrations in the control (background) and diet and in organisms using GC-MS.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently.			
Domain 6: Confounding	v / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among groups outside of the exposure.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7. Data Present	ation and Anal	lysis					
2 omain 7. Data i resolit	Metric 21:	Statistical Methods	High	Statistical analyses were described in section 2.8 and the figure captions for Figures 1 and 2 indicate that statistical analyses were done. However, the authors did not indicate the type of analyses performed for the analytical measurement of DEHP.			
	Metric 22:	Reporting of Data	High	DEHP concentrations in various larval stages and in the control (background) and diet are reported in Figures 1 and 2. They also conducted metabolomic profiling, where results are reported in the text and in Table 1.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.			
Additional Comments:	The authors	measured concentrations of DEHP in variou	is larval stages a	nd confirmed concentrations in the control (background) and diet. This form was			

used to evaluate the analytical measurements of DEHP in food and organisms (ADME).

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Diethylhexyl Phthalate

HERO ID: 5494137 Table: 1 of 5

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Study Citation:	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738.					
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Spodoptera littoralis; I	Larvae				
Health Outcome:	ADME (biotransformation)					
Chemical:	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	5494137					
Domain	Metric	Rating	Comments			

Domain	wicule	Rating	Comments
Overall Quality D	etermination	Medium	

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Study Citation: Duration: Exposure Route, Media, Path:	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Invertebrate	; Arthropods; Spodoptera littoralis; Larvae					
Health Outcome:	Mortality	Mortality					
Chemical: HERO ID:	5494137	yi phthalate (DEHP)					
 	5151157	Matria	Dating	Commanta			
Domain 1: Test Substand	ce.	Metric	Katilig	Comments			
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.			
	Metric 2:	Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verify it analytically.			
	Metric 3:	Test Substance Purity	Low	Purity was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High Madiana	Solvent control (0.5% ethanol) was used.			
	Metric 5:	Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environ- ment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2: Exposure Ch	aractorization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.			
	Metric 8:	Consistency of Exposure	Medium	It is unclear how the diet was spiked with the test material.			
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS). Methods were described in section 2.2.			
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from 4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 10 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.			
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PUBLIC RELEASE DRAFT May 2025

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Study Citation: Duration: Exposure Route,	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial: Food/Diet; Dietary					
Media, Path:						
Taxa, Species, Age:	Invertebrate	; Arthropods; Spodoptera littoralis; Larvae				
Health Outcome:	Mortality					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5494137					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).		
	,					
Domain 5: Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not suffi- ciently reported to evaluate whether differences occurred between control and exposed populations.		
	Metric 17:	Outcome Assessment Methodology	High	Mortality was reported from the 4th instar to the formation of the chrysalis.		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently.		
Domain 6: Confounding	g / Variable Co	ntrol				
Domain 0. Comountaing	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.		
Domain 7. Data Present	tation and Anal	lysis				
2 chian / Pau Prosent	Metric 21:	Statistical Methods	High	Statistical analyses were described in section 2.8. Mortality rates during larval and pupa stages were analyzed with a logistic regression test.		
	Metric 22:	Reporting of Data	High	Cumulative mortality was reported for all treatments and control with a measure of variability (Figure 3).		
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.		
Additional Comments:	This form is	specific to the results for cumulative mortal	ity.			
	4 D-4-		Л			
Overall Quali	ty Deterr	nination	weatum			

Study Citation: Duration: Exposure Route, Media, Path:	 Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Sia (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Sp littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary 							
Taxa, Species, Age:	Invertebrate;	Arthropods; Spodoptera littoralis; Larvae						
Chemical:	Di-ethylhexy	Behavioral Di-ethylbeyyl phthalate (DEHP)						
HERO ID:	5494137	5494137						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.				
	Metric 2:	Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.				
	Metric 3:	Test Substance Purity	Low	Purity was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Solvent control (0.5% ethanol) was used.				
	Metric 5:	Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environ- ment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 2: Exposure Ch	arastarization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.				
	Metric 8:	Consistency of Exposure	Low	Unclear how the diet was spiked with the test material.				
	Metric 9:	Measurement of Test Substance	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2				
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.				
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Diethylhexyl Phthalate

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Study Citation:	Aviles, A., I (2019). Effe littoralis. Ch	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738.						
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days							
Exposure Route,	Terrestrial; Food/Diet; Dietary							
Media, Path:								
Taxa, Species, Age:	Invertebrate	; Arthropods; Spodoptera littoralis; Larvae						
Health Outcome:	Behavioral							
Chemical: HERO ID:	Di-ethylhexy 5494137	yl phthalate (DEHP)						
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not suffi- ciently reported to evaluate whether differences occurred between control and exposed populations.				
	Metric 17:	Outcome Assessment Methodology	High	The food consumption behavior results were shown in Figure 6.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	lysis						
	Metric 21:	Statistical Methods	High	Statistical analyses were described in detail in section 2.8. Food consumption was ana- lyzed with the Dunnet's Test.				
	Metric 22:	Reporting of Data	Medium	Food consumption data was shown in Figure 6. Each data point represents a mean value without measures of variability.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.				
Additional Comments:	Authors exa spanned the	mined the effects of DEHP on food consum duration of development, and adult stage is a	ption behavior about 5-10 days	of Spodoptera littoralis. The exposure duration was not described explicitly but				

Overall Quality Determination

Medium

Study Citation: Duration:	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days							
Exposure Route,	Terrestrial; Food/Diet; Dietary							
Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function Di-ethylbexyl phthalate (DEHP)							
HERO ID:	5494137							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.				
	Metric 2:	Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.				
	Metric 3:	Test Substance Purity	Low	Purity was not reported.				
Domain 2: Test Design								
6	Metric 4:	Negative Controls	High	Solvent control (0.5% ethanol) was used.				
	Metric 5:	Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.				
	Metric 8:	Consistency of Exposure	Medium	Unclear how the diet was spiked with the test material.				
	Metric 9:	Administration Measurement of Test Substance	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2.				
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Test Organis	m							
<i>a</i>	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.				
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Diethylhexyl Phthalate

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Study Citation: Duration: Exposure Route, Media, Path:	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age:	Invertebrate;	Arthropods; Spodoptera littoralis; Larvae					
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Cell signa	aling/function				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	5494137						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors reported number of organism for the various endpoints in the figure cap- tions. For the hemolymphatic analysis, authors reported $n = 7$ to 12. For gene expression, the authors reported $n = 6$ for each condition in the figure caption. It is unclear if there were replicates.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not suffi- ciently reported to evaluate whether differences occurred between control and exposed populations.			
	Metric 17:	Outcome Assessment Methodology	High	The mechanistic endpoints examined in this study were reported and sensitive to DEHP exposure. They included: hemolymphatic ecdysteroid titers and expression levels of ecdysteroid response genes (reported in Figures 7 and 8).			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical analyses were described in section 2.8. Hemolymphaticconcentrations of ecdysteroids and DEHP were comparedusing aWilcoxon sum of rank test. qPCR results were analyzed withan ANOVA and Student t-test. Metabolomic results were analyzedusing ANOVA and Student t-test.			
	Metric 22:	Reporting of Data	High	For results for the hemolymphatic ecdysteroid titers and expression levels of ecdysteroid response genes are reported in figures 7 and 8.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained			
Additional Comments:	The authors ecdysteroid	examined several mechanistic endpoints incl response genes.	uding whether l	DEHP treatments affect hemolymphatic ecdysteroid titers and expression levels of			

Overall Quality Determination

Medium

Study Citation:	Aviles, A., H	Boulogne, I., Durand, N., Maria, A., Cordein	ro, A., Bozzola	n, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D.		
Duration: Exposure Route, Media. Path:	(2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodor littoralis. Chemosphere 215:725-738. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary					
Taxa. Species. Age:	Invertebrate:	Arthropods: Spodoptera littoralis: Larvae				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5494137					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.		
	Metric 2:	Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.		
	Metric 3:	Test Substance Purity	Low	Purity was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Solvent control (0.5% ethanol) was used.		
	Metric 5:	Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environ- ment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
	, . , .					
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated		
	Metric 8:	Consistency of Exposure	Low	Unclear how the diet was spiked with the test material		
	Metric 9:	Measurement of Test Substance	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2		
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.		
	Continued on next page					

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Diethylhexyl Phthalate

Study Citation: Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth Spodoptera littoralis. Chemosphere 215:725-738. Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Exposure Route, Terrestrial: Food/Diet: Dietary Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Spodoptera littoralis; Larvae Development/Growth **Health Outcome: Chemical:** Di-ethylhexyl phthalate (DEHP) **HERO ID:** 5494137 Domain Metric Rating Comments Number of Organisms and Low The authors report under the figure that n=35 larvae were used for each treatment, but it Metric 15: is unclear if each individual was treated as a replicate, or if the 35 animals were spread Replicates per Group across technical replicates (chambers, jars, etc). Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations. Metric 17: Outcome Assessment Methodology High The developmental/growth outcomes reported include: the days to pupation (post embryonic development time), and weight of the larvae (Figures 4 and 6). Metric 18: Consistency of Outcome High Outcomes were assessed consistently. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There were no reported differences among groups outside of the exposure. Design and Procedures Outcomes Unrelated to Exposure Medium Metric 20: There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical analyses were described in detail in section 2.8. For development and growth outcomes: Sex-ratio was analyzed by comparison of proportions with a bilateral test (Monte Carlo method). Larval body mass changes and food consumption were analyzed with the Dunnet's Test. Durations of larval and pupal developmental stages were analyzed by one-way ANOVA. Metric 22: Reporting of Data Low For the weight results, authors reported data in a figure as the mean weight without measures of variability. Metric 23: Explanation of Unexpected Outcomes High Outcomes were satisfactorily explained Additional Comments: Authors examined the effects of DEHP on post embryonic development of Spodoptera littoralis. The exposure duration was not described explicitly but spanned the duration of development, and adult stage is about 5-10 days. Development/growth outcomes examined in this study include time to pupation (post embryonic development) and weight.

Overall Quality Determination Medium

Duration: Contaminants of emerging concern by use of the Allium cepa test. Mutation Research /4 Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)	43(1-2):20-24.						
	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Terrestrial; Water; Root uptake	Terrestrial; Water; Root uptake						
Media, Path:							
Taxa, Species, Age: Vegetation; Vascular Plants; <i>Allium cepa</i> ; Not Applicable (e.g., fungi or algae studies) o	or Not Reported						
Health Outcome: Mechanistic-Genotox (including DNA repair)							
Chemical: Di-ethylhexyl phthalate (DEHP)							
HERO ID: 1249401							
Domain Metric Rating	Comments						
Domain 1: Test Substance							
Metric 1: Test Substance Identity High The test substance w	vas identified by name and CASRN.						
Metric 2: Test Substance Source Low The source was reported forming laboratory.	orted but the test substance was not analytically verified by the per-						
Metric 3: Test Substance Purity Low Purity and grade of the second secon	test substance were not reported.						
Domain 2: Test Design							
Metric 4: Negative Controls High Study authors report	ted using an appropriate concurrent negative control group.						
Metric 5: Negative Control Response High The biological response	onse of the negative control was adequate.						
Metric 6: Randomized Allocation Low Researchers did not	report how organisms were allocated to study groups.						
Domain 3: Exposure Characterization							
Metric 7: Experimental System/Test Media Low The study provided Preparation study.	only limited details on the measures taken to appropriately prepare and concentrations of test substance were not measured during the						
Metric 8: Consistency of Exposure High Details of exposure Administration consistently across s	administration were reported and exposures were administered study groups.						
Metric 9: Measurement of Test Substance Low Exposure concentrat	tions were not measured.						
Metric 10: Exposure Duration and Frequency High The duration of exposure Study type.	osure and exposure frequency were reported and appropriate for the						
Metric 11: Number of Exposure Groups/ High The number of expo	osure groups and spacing of exposure levels were adequate to elicit a						
Spacing of Exposure Levels response in the mech	nanistic effects studied.						
observed in the solvent concent	rent control.						
Domain 4: Test Organism							
Metric 13: Test Organism Characteristics High The source of onion	bulbs and the weight range (15-30g) were reported.						
Metric 14: Acclimatization and Pretreatment High The pretreatment co Conditions	onditions were the same for control and exposed bulbs.						
Continued on next page							

Diethylhexyl Phthalate

		conti	nued from p	previous page					
Study Citation:	Herrero, O., contaminant	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the Allium cepa test. Mutation Research 743(1-2):20-24.							
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)								
Exposure Route,	Terrestrial; V	Terrestrial; Water; Root uptake							
Media, Path:	Vagatation	Vescular Plants: Allium cong: Not Applicab	la (a g fung	ri or algea studios) or Not Dapartad					
Taxa, Species, Age: Hoolth Outcomo:	Mechanistic	Genotox (including DNA repair)	ne (e.g., rung	gi or argae studies) or Not Reported					
Chemical.	Di-ethylbexy	l nhthalate (DFHP)							
HERO ID:	1249401	r philiada (DEM)							
Domain		Metric	Rating	Comments					
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 bulbs were used for each concentration and control group. The use of replicates was not explicitly reported but in the data analysis section, it was stated that each data point represents mean \pm standard deviation of three independent experiments. Assumption was made that the details in the experimental procedures section applies to the mitotic index and chromosome aberrations experiments.					
Domain 5: Outcome As	sessment								
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Bulbs were grown in the dark at a constant temperature of 25 ± 0.5 oC in an incubator. Bulbs were kept in glass receptacles filled with filtered tap water, which was aerated continuously. It was unclear whether these conditions (incubator at 25 oC) were main- tained during exposure.					
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies for determining mitotic index and scoring of mi- cronuclei and chromosome aberrations were reported.					
	Metric 18:	Consistency of Outcome Assessment	High	Mechanistic endpoints were measured after 48 hours of exposure and appears to be consistently done across treatment groups.					
Domain 6: Confounding	y / Variable Cou	atrol							
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.					
Domain 7: Data Present	ation and Anal	vsis							
2 chiuni / Duu 11050m	Metric 21:	Statistical Methods	High	Statistical methods were clearly reported.					
	Metric 22:	Reporting of Data	High	Cytogenetic alterations were given as mean \pm standard deviation for each treatment group and control in Table 3. Statistical significance was also presented.					
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.					
Additional Comments:	Mitotic activ was used to a	ity in root meristems was first evaluated a conduct subsequent experiments to determine	nd based on	the results, the concentrations that produced a mitotic index above 50% of the control tic alterations in meristematic cells.					

Overall Quality Determination	High

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Study Citation:	Herrero, O.,	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the Allium cena test. Mutation Research 743(1-2):20.24						
Duration: Exposure Route, Media Path:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Water; Root uptake							
Taxa, Species, Age:	Vegetation; Vascular Plants; Allium cepa; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Developmer	nt/Growth						
Chemical:	Di-ethylhex	yl phthalate (DEHP)						
Domain	1249401	Metric	Rating	Comments				
Domain 1: Test Substa	nce	Mette	Rating	Comments				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name and CASRN.				
	Metric 2:	Test Substance Source	Low	The source was reported but the test substance was not analytically verified by the per- forming laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported. Test purity was described as the "highest grade commercially available".				
Domain 2: Test Desigr	1							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure C	haracterization							
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and concentrations of the test substance were not measured during the study.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of exposure groups and spacing of exposure levels were not conducive to the purpose of the study. EC50 values could not be determined.				
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate. No effects on biological responses were observed in the solvent control.				
Domain 4: Test Organi	ism							
c	Metric 13:	Test Organism Characteristics	High	The source of onion bulbs and the weight range (15-30g) were reported.				
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were the same for control and exposed bulbs.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Five bulbs were used for each concentration and control group. The use of replicates was not explicitly reported, but in the data analysis section it was stated that each data point represents mean \pm standard deviation of three independent experiments				

Continued on next page ...

Diethylhexyl Phthalate

		con	tinued from previou	s page			
Study Citation: Duration: Exposure Route, Media, Path:	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the Allium cepa test. Mutation Research 743(1-2):20-24. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Water; Root uptake						
Iaxa, Species, Age: Health Outcome:	Developmen	vascular Plants; Allium cepa; Not Applicable (e.g., fungi or algae su	idies) or Not Reported			
Chemical: HERO ID:	Di-ethylhexy 1249401	yl phthalate (DEHP)					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Bulbs were grown in the dark at a constant temperature of 25 ± 0.5 C in an incubator. Bulbs were kept in glass receptacles filled with filtered tap water, which was aerated continuously. It was unclear whether these conditions (incubator at 25C) were main- tained during exposure.			
	Metric 17:	Outcome Assessment Methodology	Low	The root length measurement method was not clearly reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.			
	Metric 18:	Consistency of Outcome Assessment	High	Root length was measured after 72 hours of exposure and appears to be consistent across treatment groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.			
Domain 7: Data Present	tation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Statistical methods were clearly reported.			
	Metric 22:	Reporting of Data	High	Mean root length data \pm standard deviation (presented as percentage of control values) was given for each treatment group and control.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	A dose-depe	endent effect of DEHP on growth (via measurer	nent of root length) c	ould not be established and EC50 values could not be determined.			

Overall Quality Determination

Uninformative

	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate.							
Dunations	Frontiers of Environmental Science & Engineering 9(2):259-268.							
Duration: Exposure Doute	Terrestrial: Soil: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)							
Media Path.	renestral, son, not determined by study autions (i.e., enemiear of merest in exposure water, but unable to determine exact uptake roue)							
Taxa, Species, Age:	Vegetation: V	Vascular Plants: Allium cepa: Embryo						
Health Outcome:	Reproductive	e/Teratogenic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	2915866							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	•							
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%				
Domain 2: Test Design	X . ·		TT: 1					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	quate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3 [,] Exposure Char	racterization							
Domain 5. Exposure char	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in				
		Preparation	8	adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
		Administration	-					
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose				
	M-4.:- 12.	Spacing of Exposure Levels	NT/A	response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.				
Domain 4: Test Organism								
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.				
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-				
		Replicates per Group		ize toxicological effects.				

Domain 5: Outcome Assessment

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PUBLIC RELEASE DRAFT **May 2025**

Diethylhexyl Phthalate

Study Citation:

Duration:

Media, Path:

Chemical:

... continued from previous page Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Exposure Route**, Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; Allium cepa; Embryo Taxa, Species, Age: Health Outcome: Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 2915866

HERO ID:	2915866			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Present	ation and Anal	vsis		
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This form re	presents the germination rate results presen	ted in Table	1 for Allium cepa with DEHP exposure.

Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Avena sativa; Embryo				
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Photosy	nthesis			
Chemical:	Di-ethylhexy	vl phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Hıgh	Chemical purity reported as 99.6%		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in		
		Preparation	ingn	adequate detail		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response seeds exposed via soil		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds		
	Metric 15:	Conditions Number of Organisms and Paplicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects		
		Replicates per Gloup				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		conti	nued from p	previous page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	s (0-96h)			
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Avena sativa; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	MDA''MDA	contents indicated distinctive resilience of	ryegrass, alf	alfa and onion under DnBP and DEHP stress compared with the other testspecies."			
Overall Qualit	ty Deterr	nination	High				

PUBLIC RELEASE DRAFT

Study Citation: Duration: Exposure Route,	Ma, T., Teng Frontiers of I Overall Dura Terrestrial; S	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Avena sativa; Embryo						
Health Outcome:	Developmen	t/Growth						
Chemical:	D1-ethylhexy	I phthalate (DEHP)						
Domain	2913800	Matria	Dating	Commonts				
Domain 1: Test Substan	re .	Metric	Katilig	Comments				
Domain 1. Test Substant	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.6%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose				
		Spacing of Exposure Levels	0	response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds were exposed via soil.				
Domain 4: Test Organisi	m							
-	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.				
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.				

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Diethylhexyl Phthalate

		contii	ued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)			
Exposure Route,	Terrestrial; S	soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:		, , , , , , , , , , , , , , , , , , ,					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Avena sativa; Embryo					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."						

High

Overall Quality Determination

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Study Citation: Duration:	Ma, T., Teng Frontiers of Overall Dura	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route, Modia Dathy	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path: Taya Species Age:	Vegetation	Vascular Plants: Avana sativa: Embryo					
Health Outcome	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	/l phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade- quate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
		т					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 2915866 Table: 3 of 3

		conti	nued from p	revious page			
Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.						
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	boil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Avena sativa; Embryo					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethvlhexy	vl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form re	presents the germination rate results preser	nted in Table	1 for Avena sativa with DEHP exposure.			
Overall Qualit	y Detern	nination	High				

Study Citation:	Wu, Z., Zhai	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant Benincasa hispida and its use for						
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; A	verall Duration: > 21 days; Exposure Duration: > 21 days prrestrial; Air; Dermal (topical application)						
Taxa, Species, Age:	Vegetation; V	egetation; Vascular Plants; Benincasa hispida; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biot	ransformation)						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	2215486							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by accepted name [Di(2-ethylhexyl) phthalate (DEHP)].				
	Metric 2:	Test Substance Source	Low	The source was not reported.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99%.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	Low	A baseline value of no DEHP would be assumed, but no concurrent negative control was used.				
	Metric 5:	Negative Control Response	Low	The biological response of a negative control group was not reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization		_					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported, and exposures were administered consistently for the one exposure concentration.				
	Metric 9:	Measurement of Test Substance	Low	Plant material was measured for DEHP, but not the air concentration.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was sufficient to detect plant uptake.				
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.				
		Spacing of Exposure Levels						
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation.				
Domain 4: Test Organisi	m							
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Medium	Test conditions were similar before and during the exposure.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Plant material was used as replicates.				
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.				
		Conf	inued on nex	st page				
Continued on next page								

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		conti	nued from p	revious page			
Study Citation:	Wu, Z., Zha	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant Benincasa hispida and its use for					
	lowering DE	lowering DEHP content of intercropped vegetables. Journal of Agricultural and Food Chemistry 61(22):5220-5225.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Terrestrial; A	Air; Dermal (topical application)					
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; <i>Benincasa hispida</i> ; Not A	pplicable (e.g	g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	2215486	•					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	Tissue measurement was adequate, but lack of air measurements or controls was con- cerning.			
	Metric 18:	Consistency of Outcome Assessment	Medium	There were minor differences in the timing of outcome assessment.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis of uptake was unnecessary.			
	Metric 22:	Reporting of Data	Low	No control or baseline values were provided.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	An additional experiment assessed the decrease in air contaminated with DEHP with no mention of biological effects.						
Overall Qualit	ty Detern	nination	Low				

Study Citation:	Wu, Z., Zhai	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant Benincasa hispida and its use for						
Duration: Exposure Route, Media. Path:	lowering DE Overall Dura Terrestrial; A	Vegetation; Vascular Plants; <i>Benincasa hispida</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age:	Vegetation;							
Health Outcome:	ADME (biot	transformation)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2215480							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by accepted name $[Di(2-ethylhexyl) phthalate (DEHP)]$.				
	Metric 2: Matria 2:	Test Substance Source	Low	The source was not reported.				
	Metric 5:	Test Substance Purity	High	Chemical purity was reported as 99%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	Low	No concurrent negative control was used.				
	Metric 5:	Negative Control Response	Low	The biological response of a negative control group was not reported.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.				
	Metric 8:	Consistency of Exposure	Medium	Details of exposure administration were reported, and exposures were administered consistently for the one exposure concentration				
	Metric 9:	Measurement of Test Substance	Low	Plant material was measured for DEHP, but not the air concentration.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was sufficient to detect plant uptake.				
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration was used.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via fumigation.				
Demois 4. Test Oreania								
Domain 4: Test Organis	III Metric 13.	Test Organism Characteristics	Low	The source of the test plants was not reported				
	Metric 14	Acclimatization and Pretreatment	Medium	Test conditions were similar before and during the exposure				
	Metric 15:	Conditions Number of Organisms and	Medium	The number of replicates was not reported.				
		Replicates per Group						
Domain 5: Outcome Ass	sessment							
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.				
	Metric 17:	Outcome Assessment Methodology	Low	Tissue measurement was adequate, but lack of air measurements or controls was con- cerning.				
	Metric 18:	Consistency of Outcome Assessment	Medium	There were minor differences in the timing of the outcome assessment.				
		Cont	inued on nex	t page				

Diethylhexyl Phthalate

continued from previous page							
Study Citation:	Wu, Z., Zhai lowering DE	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant Benincasa hispida and its use for lowering DEHP content of intercropped vegetables. Journal of Agricultural and Food Chemistry 61(22):5220-5225					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days	• • •			
Exposure Route,	Terrestrial; A	Air; Dermal (topical application)					
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; <i>Benincasa hispida</i> ; Not Ap	oplicable (e.g	., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Di-ethylhexyl phthalate (DEHP)						
HERO ID:	2215486	2215486					
Domain		Metric Rating Comments					
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis of uptake was unnecessary.			
	Metric 22:	Reporting of Data	Low	No control or baseline values were provided.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	An additiona	An additional experiment assessed the decrease in air contaminated with DEHP with no mention of biological effects.					

Overall Quality Determination

Low

Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	on: 0 - 4 days	(0-96h)			
Exposure Route, Media Path:	Terrestrial; S	soll; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Cucumis sativus; Embryo					
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	LOW	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 5.	Test Substance Fullty	nigii	Chemical purity was reported as 99.0%.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9.	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured			
	metric y.	Concentration	Low	Exposure concontrations were not inclusived.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Hıgh	The number of exposure groups and spacing of exposure levels were justified for a dose			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds were exposed via soil.			
Domain 4: Test Organis	m						
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.			
	M	Conditions	о М. ¹¹				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
		Repleates per Gloup					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		conti	nued from p	previous page				
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)				
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)				
Media, Path:		, , , , , , , , , , , , , , , , , , ,						
Taxa, Species, Age:	Vegetation; Vascular Plants; Cucumis sativus; Embryo							
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2915866	•						
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.				
Domain 7: Data Present	tation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	MDA,"MDA	a contents indicated distinctive resilience of	f ryegrass, al	falfa and onion under DnBP and DEHP stress compared with the other testspecies."				
Overall Quali	ty Deterr	nination	High					

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 2915866				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce		U		
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%	
Domain 2: Test Design					
6	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 2. Expansion Ch	onostanization				
Domain 5: Exposure Ch	Matria 7	Even mineratel System/Test Madia	High		
	Metric 7:	Preparation	нıgn	adequate detail	
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured	
	Wette 9.	Concentration	Low	Exposure concentrations were not measured	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose	
		Spacing of Exposure Levels		response	
	Metric 12:	Testing at or Below Solubility Limit	N/A	seeds exposed via soil	
Domain 4: Test Organis	m				
U	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source	
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health	
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups	

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Diethylhexyl Phthalate

		contii	nued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Cucumis sativus; Embryo					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Con	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."						

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Ma, T., Teng Frontiers of Overall Dura Terrestrial; S	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	,					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Cucumis sativus; Embryo				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade- quate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Continued on next page					

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PUBLIC RELEASE DRAFT May 2025

Diethylhexyl Phthalate

HERO ID: 2915866 Table: 3 of 3

		conti	nued from p	previous page			
Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268						
Duration:	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	,						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Cucumis sativus; Embryo					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form re	presents the germination rate results preser	nted in Table	1 for Cucumis sativus with DEHP exposure.			
Overall Quali	ty Deterr	nination	High				

Study Citation:	Zhang, Y., V	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the					
Duration: Exposure Route, Media. Path:	physiology a Overall Dura Terrestrial; S	Dystology and ultrastructure of cucumber seedings. Environmental Science and Pollution Research 21(2):1020-1028. Dverall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Ferrestrial; Soil; Root uptake					
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Cucumis sativus; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cytotoxie	city-Oxidative st	ress (including redox biology)-Photosynthesis			
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	1987637						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose			
		Spacing of Exposure Levels		response			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms			
	Metric 15.	Conditions Number of Organisms and	Low	The number of test organisms and/or replicates was not reported individual leaves com-			
	methe 15.	Replicates per Group	Low	prised the replicates			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
Continued on next page							

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Diethylhexyl Phthalate

HERO ID: 1987637 Table: 1 of 2

		continu	ued from previo	bus page		
Study Citation:	Zhang, Y., W physiology a	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; S	oil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Cucumis sativus; Jinchun; N	ot Applicable (e	.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cytotoxic	ity-Oxidative st	ress (including redox biology)-Photosynthesis		
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	1987637					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups		
		Assessment				
Domain 6: Confounding	, / Variable Cor	itrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Presenta	ation and Analy	ysis	-			
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group but n values were not reported		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and variability is reported. However, because it is unclear what constituted a technical replicate and how many replicates there were the reported SD is unclear.		

Additional Comments: This includes ultra structural changes which were not quantifiably assessed or analyzed for statistical signifigance

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1987637 Table: 2 of 2

Study Citation:	Zhang, Y., W	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the						
Duration: Exposure Route, Media Path	Overall Dura Terrestrial; S	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Soil; Root uptake Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age:	Vegetation; V							
Health Outcome:	Di ethylbey	Development/Growth						
HERO ID:	1987637							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Ĩ	Metric 7:	Experimental System/Test Media	Medium	Reporting omissions were unlikely to have a substantial impact on results.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported (7 days) and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate for a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.				
Domain 4: Test Organis	m							
5	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates was not reported, and individual leaves comprised the replicates.				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.				
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.				

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Diethylhexyl Phthalate

		contin	ued from previ	ous page		
Study Citation:	Zhang, Y., V physiology a	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Terrestrial; S	Soil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Cucumis sativus; Jinchun; N	ot Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	1987637					
Domain		Metric Rating Comments				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group, but sample size and the numbers of technical replicates were not reported.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and a measure of variability was reported. How- ever, it is unclear how variability was quantified (across plants, pots, how many individ- uals, etc).		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate.					
Duration: Exposure Route, Modia Pathy	Overall Dura Terrestrial; S	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; V Mechanistic- Di-ethylhexy	Vascular Plants; <i>Lolium perenne</i> ; Embryo Biomarkers (exposure and effect)-Photosy /l phthalate (DEHP)	onthesis			
Domain	2913800	Metric	Rating	Comments		
Domain 1: Test Substand	ce		8			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.6%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response. Seeds were exposed via soil.		
Domain 4: Test Organist	m					
_ shan in tost organisi	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.		
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Group		ize toxicological effects.		
Domain 5. Outages - Age	accont					
Domain 5: Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
		Conti	inued on nex	ct page		

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		conti	nued from p	previous page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Lolium perenne; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866	2915866					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	MDA''MDA	contents indicated distinctive resilience of	ryegrass, alf	alfa and onion under DnBP and DEHP stress compared with the other testspecies."			
Overall Qualit	Overall Quality Determination High						

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 2915866				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%	
Domain 2: Test Design					
0	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured	
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose	
	metric II.	Spacing of Exposure Levels	mgn	response	
	Metric 12:	Testing at or Below Solubility Limit	N/A	seeds exposed via soil	
Domain 4: Test Organisi	m				
c c	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source	
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health	
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups	

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Diethylhexyl Phthalate

		contin	nued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; <i>Lolium perenne</i> ; Embryo					
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Con	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."						

Overall Quality Determination

High

Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate.					
Duration	Frontiers of	Environmental Science & Engineering 9(2)):259-268.	(0.06h)		
Exposure Route.	Terrestrial: S	Soil: Not determined by study authors (i e	chemical of	interest in exposure water but unable to determine exact untake route)		
Media. Path:	i circouriur, c					
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Lolium perenne; Embryo				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade-		
			0	quate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
Ĩ	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.		
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Group		ובי וסגונטוספונאו פוופנוג.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
	Continued on next page					

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Diethylhexyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Lolium perenne; Embryo					
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	Di-ethylhexyl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	lycic					
Domain 7. Data Present	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form re	presents the germination rate results preser	ited in Table	1 for Lolium perenne with DEHP exposure.			
Overall Qualit	ty Detern	nination	High				

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Study Citation: Duration: Exposure Route, Media Path:	Ma, T., Teng Frontiers of Overall Dura Terrestrial; S	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome:	Vegetation; Mechanistic	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosynthesis				
HERO ID:	2915866	(DEII)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response seeds exposed via soil		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds		
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Group		ize toxicological effects		
Domain 5: Outcome As	sessment					
2 Smail 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		conti	nued from p	previous page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866	2915866					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	MDA''MDA	contents indicated distinctive resilience of	ryegrass, alf	alfa and onion under DnBP and DEHP stress compared with the other test species."			
Overall Quali	ty Deterr	nination	High				

0

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 2915866				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce		-		
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.6%.	
Domain 2: Test Design					
Domain 21 Teor Deoign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type	
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose	
	Matria 12.	Spacing of Exposure Levels	N/A	response.	
	Metric 12.	Testing at of Below Solubility Linit	IN/A	Secus were exposed via son.	
Domain 4: Test Organisi	m				
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.	
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.	
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.	

Continued on next page ...

Diethylhexyl Phthalate

		contin	nued from p	revious page		
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)		
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation; '	Vascular Plants; <i>Medicago sativa</i> ; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	2915866	2915866				
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."					

High

Overall Quality Determination

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Study Citation: Duration:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	T 7 T					
Taxa, Species, Age:	Vegetation;	Vegetation; vascular Plants; <i>Medicago sativa</i> ; Embryo				
Chemical:	Di-ethylbey	l phthalate (DEHP)				
HERO ID:	2915866	i philadae (DEIII)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		0			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade- quate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
Domain 5: Outcome As	sessment	A de sur est Trest C in l'él	TT' 1			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
Continued on next page						

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 2915866 Table: 3 of 3

		conti	nued from p	previous page		
Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Eroptiers of Environmental Science & Envi					
Duration:	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Medicago sativa</i> ; Embryo				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
	Assessment					
Domain 6: Confounding	o / Variable Co	ntrol				
Domain of Comountaing	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
	metric 19.	Design and Procedures	111511	There were no reported anterences antong the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.		
Domain 7: Data Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.		
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This form re	presents the germination rate results preser	nted in Table	1 for Medicago sativa with DEHP exposure.		
Overall Quali	ty Detern	nination	High			

Study Citation:	Deng, J., Zh	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination					
Duration	and seedling	and seedling growth. Pedosphere 27(6):1073-1082.					
Duration: Exposure Route	Terrestrial: (Terrestrial: Cell Culture Media: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media Path	Terrestriar, C	renestral, con culture media, not determined by study dutions (i.e., enclined of interest in exposure water, out undote to determine exact uptake route)					
Taxa, Species, Age:	Vegetation:	Vegetation: Vascular Plants: <i>Nicotiana tabacum</i> : cv K326: Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Reproductiv	Reproductive/Teratogenic					
Chemical:	Di-ethylhexy	Di-ethylhexyl nhthalate (DEHP)					
HERO ID:	5627041	5627041					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Reported as "guaranteed reagent grade"			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	The study reported that test containers were randomly distributed			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure on	Metric 7	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately pre-			
	incure /.	Preparation	2011	pare test concentrations. Authors reported using glassware: vials, funnels, bottles and beakers. No use of plastic vessels reported.			
	Metric 8:	Consistency of Exposure	Low	Only general methods of exposure administration were reported so assessment was			
	Matria 0.	Administration Massurement of Test Substance	Low				
	Metric 9.	Concentration	LOW	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose			
		Spacing of Exposure Levels		response			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organis	m						
s and a set of game.	Metric 13:	Test Organism Characteristics	Medium	The source of the seeds was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms			
		Conditions	8	1			
	Metric 15:	Number of Organisms and	Low	The number of test plants was not reported, three replicates used			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system (controlled chamber) were conducive to main- tenance of organism health			
		Cont	tinued on nex	xt page			

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Deng, J., Zh	ang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhan	g, S. (2017).	Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination		
	and seedling	growth. Pedosphere 27(6):1073-1082.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days			
Exposure Route,	Terrestrial; C	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	5627041	5627041				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups		
		Assessment				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures	e			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups		
Domain 7: Data Present	ation and Anal	veie				
Domain 7. Data Present	Motrio 21:	ysis Statistical Mathods	High	Statistical methods were adapted described		
	Matria 22:	Benerting of Date	High	Data for expressive related for dings were presented for each treatment and control group		
	Metric 22.	Exploration of Union and Outcomes	High LE -h	Data for exposure-related minings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained		
Additional Comments:	germination	effects				
Overall Qualit	Overall Quality Determination					

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Study Citation:	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination and seedling growth. Bedochere 27(6):1073-1082						
Duration: Exposure Route, Media Path	Overall Dura Terrestrial; C	tion: 4 - 10 days; Exposure Duration: 4 - Cell Culture Media; Not determined by stu	10 days dy authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age: Health Outcome:	Vegetation; V Developmen	/egetation; Vascular Plants; <i>Nicotiana tabacum</i> ; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth					
Chemical: HERO ID:	Di-ethylhexy 5627041	Di-ethylhexyl phthalate (DEHP) 5627041					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Reported as "guaranteed reagent grade"			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	The study reported that test containers were randomly distributed			
Domain 3: Exposure Ch	aracterization		-				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately pre- pare test concentrations. Authors reported using glassware: vials, funnels, bottles and beakers. No use of plastic vessels reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Only general methods of exposure administration were reported so assessment was difficult to determine			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 1: Test Organia							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Medium	The source of the seeds was not reported			
	Metric 13:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms			
		Conditions	6	I			
	Metric 15:	Number of Organisms and	Low	The number of test plants was not reported, three replicates used			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system (controlled chamber) were conducive to main- tenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups			
		Continued on next page					

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Diethylhexyl Phthalate

continued from previous page						
Study Citation:	Deng, J., Zha	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination				
	and seedling	growth. Pedosphere 27(6):1073-1082.				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	ly authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Nicotiana tabacum; cv K3	26; Not App	licable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	5627041					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Con Metric 19:	ntrol Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
	Medic 19.	Design and Procedures	mgn	There were no reported unreferees among the study groups in environmental conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups		
Domain 7: Data Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained		
Additional Comments:	vigor index,	length				
Overall Quality Determination			High			

Study Citation:	Jia, Z. H., Yi	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-					
Duration: Exposure Route, Media, Path:	96. Overall Dura Terrestrial; V	5. verall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days errestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vegetation; V	getation; Vascular Plants; Nicotinana tobacum; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	evelopment/Growth					
Chemical:	Di-ethylhexy	vl phthalate (DEHP)					
HERO ID:	192357						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		_				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type (7 days of exposure on filter paper).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response			
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (11.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.			
Domain 4: Test Organis	m						
en e	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.			

Domain 5: Outcome Assessment

Continued on next page ...

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 1 of 4

		conti	nued from p	previous page	
Study Citation:	Jia, Z. H., Yi	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-			
Dunation	96. O II D († 14.10.) E D († 14.10.)				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Koute, Modia Datha	Terrestriar; v	water, not determined by study authors (i.e	e., chemicai c	of interest in exposure water, but unable to determine exact uptake route)	
Toyo Species Ages	Vagatation				
Taxa, Species, Age:	Development	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Chamical	Developmen Di athylhau	Development/Growth			
	702257	Di-etnyinexyi phthalate (DEHP)			
HERO ID:	192551				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported	
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-	
		Assessment		ited	
Domain 6: Confounding	g / Variable Coi	ntrol			
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental	
		Design and Procedures			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups	
Domain 7. Data Present	ation and Anal	veis			
Domain 7. Dua Present	Metric 21:	Statistical Methods	High	Statistical methods were adequately described	
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes	
		2.1p. and of chexpected outcomes			
Additional Comments:	Growth (Dev	velopment-Slowed, Retarded, Delayed or N	lon-developm	nent, Response Site: Not reported)	
	6.	- · · · · ·	1	× • ·	
Overall Onali	t <mark>v Dete</mark> rn	nination	Low		
V VI un Vuun					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 792357 Table: 2 of 4

Study Citation:	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-						
Duration: Exposure Route, Media. Path:	96. Overall Dura Terrestrial; V	96. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V Reproductive Di-ethylhexy 792357	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 792357					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response			
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (11.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported			
		Cont	inued on nex	rt page			

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 2 of 4

		conti	nued from p	revious page		
Study Citation:	Jia, Z. H., Yi	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-				
Derections	96.	96. Oursell Duration: A 10 days, Exposure Duration: A 10 days				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Tarractrial: Water: Not determined by study outbors (i.e., shamical of interact in exposure water, but unable to determine exact untake route)					
Exposure Noure, Modia Path.	Terresular, v	Terrestrial; water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Snecies, Age:	Vegetation: V	Vascular Plants: Nicotinana tobacum: Hono	, da· Not Am	nlicable (e.g. fungi or algae studies) or Not Reported		
Health Outcome:	Reproductive	e/Teratogenic	, uu, 110t 11p	should (e.g., rungi of algae statics) of rot reported		
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	792357					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
		Assessment		ited		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
		Design and Procedures		conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups		
Domain /: Data Present	Ation and Anal	ysis Statistical Mathada	High	Charles in a surgery of a surgery in the surgery in the		
	Metric 21: Metric 22:	Reporting of Data	пign High	Statistical methods were adequately described		
	Metric 22.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	Wietife 25.	Explanation of Onexpected Outcomes	Ingn			
Additional Comments:	Germination					
Overall Qualit	ty Detern	nination	Low			

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HERO ID: 792357 Table: 3 of 4

Study Citation:	Jia, Z. H., Yi	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-				
Duration: Exposure Route, Media Path	96. Overall Dura Terrestrial; V	96. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; Developmen Di-ethylhexy	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP)				
HERO ID:	792357					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
-	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.		
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response		
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (11.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.		
Domain 4: Test Organis	m					
rest organis	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported		
		Cant	inuad on nor			
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 3 of 4

		conti	nued from p	revious page		
Study Citation:	Jia, Z. H., Yi	, J. H., Su, Y. R., Shen, H. (2011). Autotox	ic substances	s in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-		
	96. II D	96. Orangil Durations 4 10 down Francesco Durations 4 10 down				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Terrestrial; V	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	N 7 () ¹					
Taxa, Species, Age:	vegetation;	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	Development/Growth				
UEDO ID.	Di-ethylhexyl phthalate (DEHP)					
HERU ID:	192551					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
		Assessment		ited		
		4 1				
Domain 6: Confounding	g / Variable Co		T			
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Matria 20.	Design and Procedures	Madium	conditions		
	Metric 20:	Outcomes Onrelated to Exposure	Medium	There was no information in the study to suggest differences among groups		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group		
	Metric 22:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
		Explanation of Onexpected Subolics	man			
Additional Comments:	Growth (Dev	velopment-Slowed, Retarded, Delayed or N	lon-developn	nent, Response Site: Not reported)		
Overall Qualit	ty Deterr	nination	Low			

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HERO ID: 792357 Table: 4 of 4

Study Citation:	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-						
Duration: Exposure Route, Media, Path:	96. Overall Dura Terrestrial; V	96. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Reproductive Di-ethylhexy 792357	'egetation; Vascular Plants; <i>Nicotinana tobacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported eproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 92357					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type (7 days of exposure on filter paper).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response			
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (11.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 4 of 4

		conti	nued from p	revious page		
Study Citation:	Jia, Z. H., Yi	, J. H., Su, Y. R., Shen, H. (2011). Autotox	ic substances	in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-		
Duration	96. Overall Dur	ation: 4 10 days: Exposure Duration: 4 1	0 dava			
Exposure Route	Terrestrial: Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)					
Media. Path:	Terrestriar, v	refrestrat, water, not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa. Species. Age:	Vegetation: V	Vegetation: Vascular Plants: <i>Nicotinana tobacum</i> : G168: Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Reproductive	e/Teratogenic	, II			
Chemical:	Di-ethylhexy	l phthalate (DEHP)				
HERO ID:	792357					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
		Assessment		ited		
Domain 6: Confounding	g / Variable Cor	ntrol				
· · · · · ·	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
		Design and Procedures		conditions		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups		
Damain 7. Data Draamt						
Domain /: Data Present	Ation and Anal	ysis Statistical Mathada	High	Other in the standard second data with a data with a d		
	Metric 21: Metric 22:	Reporting of Data	піgli Ціар	Statistical methods were adequately described		
	Metric 22.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	Wietife 25.	Explanation of Onexpected Outcomes	Ingn			
Additional Comments:	Germination					
Overall Qualit	ty Detern	nination	Low			

Study Citation: Duration: Exposure Route, Media, Path:	Ma, T., Teng Frontiers of Overall Dura Terrestrial; S	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Raphanus sativus; Embryo					
Health Outcome:	Mechanistic	Aechanistic-Biomarkers (exposure and effect)-Photosynthesis					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Hıgh	Chemical purity was reported as 99.6%.			
Domain 2: Test Decign							
Domain 2. Test Design	Metric 4	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response. Seeds were exposed via soil.			
Domain 4: Test Organis	m						
0	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects.			
Domain 5: Outcome Ac	recement						
Domain 5: Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		conti	nued from p	previous page		
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Raphanus sativus; Embryc)			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis			
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	MDA''MDA	contents indicated distinctive resilience of	ryegrass, alf	alfa and onion under DnBP and DEHP stress compared with the other testspecies."		
Overall Qualit	Overall Quality Determination High					

Diethylhexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng Frontiers of J Overall Dura Terrestrial; S Vegetation; V Developmen Di-ethylhexy 2915866	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo Development/Growth Di-ethylhexyl phthalate (DEHP) 2915866				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce		8			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
	, . <i>.</i> .					
Domain 3: Exposure Ch	Aracterization	Engening and all Southerny (Teach Mardia	TT: -1-			
	Metric /:	Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose		
	metrie II.	Spacing of Exposure Levels	111511	response		
	Metric 12:	Testing at or Below Solubility Limit	N/A	seeds exposed via soil		
Domain 4: Test Organis	m					
0	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups		

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Diethylhexyl Phthalate

		conti	nued from p	revious page		
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Raphanus sativus; Embryo	1			
Health Outcome:	Developmen	t/Growth				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence		
				the outcome assessment.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	"DnBP and]	DEHP at a range of concentrations in the ex	perimental s	oil showed no discernible effect on the germination rate of the seven test plant species		
rudulina comments.	However, th growth in the	ey did exert effects on root elongation, se e evaluation of the phytotoxicity of PAE co	edling grow mpounds."	th and biomass to different extents, indicating the potential applicability of seedling		

High

Overall Quality Determination

Page 936 of 958
Study Citation: Duration:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo					
Health Outcome:	Reproductive	e/ leratogenic					
HFRO ID.	2915866	(DEHP)					
Domain	2913000	Matria	Dating	Commente			
Domain 1: Test Substan	ce	Metric	Kating	Comments			
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the			
				performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%			
Domain 2: Test Design							
c c	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade- quate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.			
Domain 4: Test Organis	m						
-	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome As	sessment		TT:-h				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 2915866 Table: 3 of 3

		conti	nued from p	previous page				
Study Citation:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268							
Duration:	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)							
Exposure Route,	Terrestrial; S	Soil: Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)				
Media, Path:	,	renestina, son, not determined of study admors (ne., chemical of merest in exposure water, but anable to determine exact uptake fould)						
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Raphanus sativus; Embryo						
Health Outcome:	Reproductiv	e/Teratogenic						
Chemical:	Di-ethylhexyl phthalate (DEHP)							
HERO ID:	2915866							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
	Assessment							
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.				
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	This form re	presents the germination rate results preser	nted in Table	1 for Raphanus sativus with DEHP exposure.				
Overall Quali	ty Deterr	nination	High					

Study Citation:	Ma, T., Teng	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate.					
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; S	Verall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Perestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Triticum aestivum; Embryo	0				
Health Outcome:	Developmen	t/Growth					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.6%.			
Domain 2: Test Design							
8	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response. Seeds were exposed via soil.			
Domain 4. Test Organist	m						
2 omain 1. Test Organisi	Metric 13	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects.			
Domain 5: Outcome Age	assmant						
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
	Continued on next page						

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Diethylhexyl Phthalate

		contir	nued from p	revious page				
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Vegetation; Vascular Plants; Triticum aestivum; Embryo							
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	2915866	2915866						
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding / Variable Control								
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate there were differences among groups that could influ- ence the outcome assessment.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."							

Overall Quality Determination	High	
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Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	(0-96h)				
Exposure Route,	Terrestrial; S	oil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Vegetation; V	Vegetation; Vascular Plants; Triticum aestivum; Embryo						
Health Outcome:	Reproductive	Reproductive/Teratogenic						
Chemical:	Di-ethylhexy	l phthalate (DEHP)						
HERO ID:	2915866							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.				
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade- quate.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
-	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.				
Domain 4: Test Organis	m							
0	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed seeds.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.				
Domain 5: Outcome Ass	sessment		TT' 1					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
Continued on next page								

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Diethylhexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 2915866 Table: 2 of 3

		conti	nued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Triticum aestivum; Embryo	D				
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Di-ethylhexy	l phthalate (DEHP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form re	presents the germination rate results preser	nted in Table	1 for Triticum aestivum with DEHP exposure.			
Overall Qualit	ty Detern	nination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosynthesis Di-ethylhexyl phthalate (DEHP) 2915866					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only		
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	Domain 3: Exposure Characterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were justified for a dose		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.		
Domain 4: Test Organis	m					
8	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.		
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed seeds.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.		
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. outcomes were assessed consistently across study groups.		

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 3 of 3

		conti	nued from p	previous page			
Study Citation:	Ma, T., Teng Frontiers of	Ma, T., Teng, Y., Christie, P., Luo, Y. (2015). Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science & Engineering 9(2):259-268					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Triticum aestivum; Embryo	D				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2915866	2915866					
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	MDA, chlorophyll, carotinoid""MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other testspecies."						
Overall Qualit	ty Deterr	nination	High				

Diethylhexyl Phthalate

Study Citation:	Gao, M., Do	ong, Y., Liu, Y., Song, Z. (2018). Photosynth	netic and antioxic	lant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the				
	soil. Chemo	soil. Chemosphere 209:258-267.						
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported							
Exposure Route,	Terrestrial; Soil; Root uptake							
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; Triticum aestivum; Jingqia	ng 8; Not Applica	able (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mechanistic	-Oxidative stress (including redox biology)-	-Photosynthesis					
Chemical:	Di-ethylhex	yl phthalate (DEHP)						
HERO ID:	5493185							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The test substance was clearly identified. Chemical name and CASRN were provided.				
	Metric 2:	Test Substance Source	Low	DEHP was purchased from Lark Technology Co., Ltd. (Beijing, China). The test sub-				
				stance identity was NOT analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	Hıgh	Purity was 96.8%.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4.	Negative Controls	High	Methanol was used as a solvent and a methanol control was used				
	Metric 5:	Negative Control Response	High	The biological responses of the control group were reported and normal				
	Metric 6:	Randomized Allocation	Medium	Seeds were placed at random				
	incure of	Rundoninzed Philocution	meanum					
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media	Low	Soil was prepared by mixing such that final concentrations were 10, 20, and 40 mg/kg				
		Preparation		(dry weight). Methanol was evaporated. Concentrations were not verified				
	Metric 8:	Consistency of Exposure	Medium	Exposure administration was reported, but because concentrations were not verified it is				
		Administration		unclear how consistent exposures were across groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured				
		Concentration						
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was from seedling to booting stage of wheat, but it is unclear how long this is.				
	Metric 11:	Number of Exposure Groups/	High	Exposure concentrations were mixed and estimated to be: 10, 20, and 40 mg/kg (dry				
	M + 1 10	Spacing of Exposure Levels	3.7.1.4	weight).				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil.				
Domain 4. Test Organis	m							
Domain 4. Test Organis	Metric 13	Test Organism Characteristics	High	The source of the wheat seeds and rationale for selection of an arricultural grop species				
	metric 13.	Test organism characteristics	mgn	was described.				
	Metric 14:	Acclimatization and Pretreatment	High	Seeds were sterilized and washed prior to seeding and treatment environmental condi-				
		Conditions	8	tions were kept constant throughout the experiment.				
	Metric 15:	Number of Organisms and	Medium	20 seeds were placed in each pot and 3 replicate pots were used per treatment group				
		Replicates per Group		_ _				

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

HERO ID: 5493185 Table: 1 of 2

ng, Y., Liu, Y., Song, Z. (2018). Photosynth sphere 209:258-267. titon: Not-reported; Exposure Duration: No oil; Root uptake Vascular Plants; <i>Triticum aestivum</i> ; Jingqiar Oxidative stress (including redox biology)- ol phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	netic and antioxid ot-reported ng 8; Not Applic Photosynthesis Rating High Medium Low	dant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the able (e.g., fungi or algae studies) or Not Reported <u>Comments</u> Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
ition: Not-reported; Exposure Duration: Notoil; Root uptake Vascular Plants; <i>Triticum aestivum</i> ; Jingqiar Oxidative stress (including redox biology)- I phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	ng 8; Not Applic Photosynthesis Rating High Medium Low	able (e.g., fungi or algae studies) or Not Reported Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Availia Root uptake Vascular Plants; <i>Triticum aestivum</i> ; Jingqiar Oxidative stress (including redox biology)- el phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	ng 8; Not Applic Photosynthesis Rating High Medium Low	able (e.g., fungi or algae studies) or Not Reported Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Vascular Plants; <i>Triticum aestivum</i> ; Jingqiar Oxidative stress (including redox biology)- I phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	ng 8; Not Applic Photosynthesis Rating High Medium Low	able (e.g., fungi or algae studies) or Not Reported Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Vascular Plants; <i>Triticum aestivum</i> ; Jingqiar Oxidative stress (including redox biology)- I phthalate (DEHP) <u>Metric</u> Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	ng 8; Not Applic Photosynthesis Rating High Medium Low	Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Oxidative stress (including redox biology)- l phthalate (DEHP) Metric Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	Photosynthesis Rating High Medium Low	Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Metric Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	Rating High Medium Low	Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Metric Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	Rating High Medium Low	Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Metric Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	Rating High Medium Low	Comments Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	High Medium Low	 Environmental conditions and soil chemistry were reported and conducive to the health of the plants. The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. 			
Outcome Assessment Methodology Consistency of Outcome Assessment	Medium Low	The "mechanistic" endpoints examined included photosynthetic parameters and chloro- phyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT. MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
Consistency of Outcome Assessment	Low	The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.			
ntrol					
Confounding Variables in Test Design and Procedures	Low	Background concentrations on contaminants in the soil were not measured. Environ- mental conditions of the treated plots over time were monitored but results were not reported.			
Outcomes Unrelated to Exposure	Medium	There was no information to suggest effects due to other variables, but background concentrations of contaminants in the soil were not measured.			
ysis					
Statistical Methods	High	Sufficient data are provided to conduct an statistical analysis if needed			
Reporting of Data	High	Means and std error are reported for control and treatment groups			
Explanation of Unexpected Outcomes	High	Damage caused by DEHP was mitigated by enzymatic activity in response to exposure.			
The authors exposed wheat to three concentrations of DEHP mixed with the soil, starting as seedlings and through to the reproductive stage. They measured kernal weight as well as various endpoints to quantify effects on photosynthesis, induction of reactive oxygen species, and response of the antioxidant defense system. Endpoints were examined at three stages: seedling, jointing, and booting. The exact exposure duration is unclear and concentrations were not measured.					
	Design and Procedures Outcomes Unrelated to Exposure ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes exposed wheat to three concentrations of DF t as well as various endpoints to quantify m. Endpoints were examined at three stage	Design and Procedures Outcomes Unrelated to Exposure Medium ysis Statistical Methods High Reporting of Data High Explanation of Unexpected Outcomes High exposed wheat to three concentrations of DEHP mixed with the tas well as various endpoints to quantify effects on photocom. Endpoints were examined at three stages: seedling, join Lemontation			

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Study Citation: Duration: Exposure Route,	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Soil; Root uptake							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Developmen Di-ethylhexy 5493185	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Jingqiang 8; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP) 5493185						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The test substance was clearly identified. Chemical name and CASRN were provided. DEHP was purchased from Lark Technology Co., Ltd. (Beijing, China). The test sub- stance identity was NOT analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Purity was 96.8%.				
Domain 2: Test Design								
Domain 2. Test Design	Metric 4.	Negative Controls	High	Methanol was used as a solvent and a methanol control was used				
	Metric 5:	Negative Control Response	High	The biological responses of the control group were reported and normal.				
	Metric 6:	Randomized Allocation	Medium	Seeds were placed at random.				
Demain 2: Errorenne Ch								
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	Sail was propaged by mixing such that final concentrations were 10, 20, and 40 mg//cg				
	Weute 7.	Preparation	LOw	(dry weight). Methanol was evaporated. Concentrations were not verified.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure administration was reported, but because concentrations were not verified it is unclear how consistent exposures were across groups.				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The exposure duration was from seedling to booting stage of wheat, but it is unclear how long that was.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were mixed and estimated to be: 10, 20, and 40 mg/kg (dry weight).				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.				
Domain 4: Test Organis	Motri - 12	Test Organism Characteristics	11:-1-					
	Metric 13:	Test Organism Characteristics	High	The source of the wheat seeds and rationale for selection of an agricultural crop species was described.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Seeds were sterilized and washed prior to seeding, and treatment environmental condi- tions were kept constant throughout the experiment.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Twenty seeds were placed in each pot and three replicate pots were used per treatment group.				
Domain 5: Outcome Ag	recement							
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions and soil chemistry were reported and conducive to the health of the plants.				
	Metric 17:	Outcome Assessment Methodology	Medium	The growth endpoint examined was the dry weight of the wheat kernel.				
Continued on next page								

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HE

		contin	ued from previ	ous page				
Study Citation:	Gao, M., Do soil. Chemo	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267						
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported							
Exposure Route,	Terrestrial; S	Terrestrial; Soil; Root uptake						
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; Triticum aestivum; Jingqian	g 8; Not Applica	able (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	5493185							
Domain		Metric	Rating	Comments				
	Metric 18: Consistency of Outcome Medium It is unclear when the wheat kernels were collected and measured (after h of exposure for each treatment).							
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Background concentrations on contaminants in the soil were not measured. Environ- mental conditions of the treated plots over time were monitored but results were not reported.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest effects due to other variables, but background concentrations of contaminants in the soil were not measured.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Sufficient data are provided to conduct a statistical analysis if needed.				
	Metric 22:	Reporting of Data	High	Means and standard error were reported for control and treatment groups.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Damage caused by DEHP was mitigated by enzymatic activity in response to exposure.				
Additional Comments:	The authors exposed wheat to three concentrations of DEHP mixed with the soil, starting as seedlings and through to the reproductive stage. They measured kernel weight as well as various endpoints to quantify effects on photosynthesis, induction of reactive oxygen species, and response of the antioxidant defense system. Endpoints were examined at three stages: seedling, jointing, and booting. The exact exposure duration is unclear and concentrations were not measured.							

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Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	fluorescence Overall Dura Terrestrial; S Vegetation; D Developmen Di-ethylhex 3350318	Gao, M., Qi, Y., Song, W., Xu, H. (2016). Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings. Chemosphere 151:76-83. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Soil; Root uptake Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP) 3350318						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#.				
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 96.8%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in				
	Methe 7.	Preparation	Ingn	adequate detail. Test solutions were replenished daily.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the bio- logical response of the solvent control was acceptable.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms (8 seedlings per concentration) were reported and suffi- cient to characterize toxicological effects. Replicates were not reported but it was stated that the experiment was repeated five times.				

Diethylhexyl Phthalate

HERO ID: 3350318 Table: 1 of 2

		contin	ued from previ	ious page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Gao, M., Qi, Y., Song, W., Xu, H. (2016). Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings. Chemosphere 151:76-83. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Soil; Root uptake Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Di-ethylhexyl phthalate (DEHP) 3350318						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health."The experiments were conducted in an artificial climate chamber. The seedlings were cultured under a cycle of 12-h days at 25 ± 1 oC and 12-h nights at 20 ± 1 oC at 60% relative humidity level."			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodologies for plant height, fresh weights of shoots and roots and dry weight of shoots and roots were reported but not in sufficient detail.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
·	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported, but these are unlikely to have a substantial impact on results. The experiments were conducted in an artificial climate chamber.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but calculations and measures of significance were not provided, so no conclusions about a dose response could be made. Results of multi- ple comparisons were not provided.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group (Table 1).			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	The author's incorporatin	discussion of the effects of DBP and DEH g any discussion of statistical significance. R	P on growth indesults of multip	dices of wheat seedlings was not clear, and their conclusions were made without ole comparisons were not provided.			

Overall Quality Determination

Medium

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HERO ID: 3350318 Table: 2 of 2

Study Citation:	Gao, M., Qi, Y., Song, W., Xu, H. (2016). Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll						
Duration	fluorescence	fluorescence of wheat seedlings. Chemosphere 151:/6-83. Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days					
Duration: Exposure Route	Terrestrial: S	Terrestrial: Soil: Root untake					
Media Path.	Terrestriar, Son, Root uptake						
Taxa, Species, Age:	Vegetation: V	Vascular Plants: Triticum sp: Jinnong 7: No	ot Applicable	(e.g. fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic-	-Photosynthesis	or ripplicuoie	(0.5., rungi of ulgue studies) of not reported			
Chemical:	Di-ethylhexy	/l phthalate (DEHP)					
HERO ID:	3350318						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 96.8%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. Test solutions were replenished daily.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the bio- logical response of the solvent control was acceptable.			
Domain 4: Test Organis	m						
2 smain 1. rost organis	Metric 13.	Test Organism Characteristics	Hioh	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms			
		Conditions		In predetation conclusion were the sume for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms (eight seedlings per concentration) were reported and sufficient to characterize toxicological effects. Replicates were not reported, but it was			
				stated that the experiment was repeated five times.			
Domain 5: Outcome Ass	sessment						
Continued on next page							

Diethylhexyl Phthalate

HERO ID: 3350318 Table: 2 of 2

		conti	nued from p	revious page				
Study Citation:	Gao, M., Qi, fluorescence	Gao, M., Qi, Y., Song, W., Xu, H. (2016). Effects of di-n-butyl phthalate and di (2-ethylhexyl) phthalate on the growth, photosynthesis, and chlorophyll fluorescence of wheat seedlings. Chemosphere 151:76-83.						
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days					
Exposure Route,	Terrestrial; S	Terrestrial; Soil; Root uptake						
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; No	t Applicable	(e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mechanistic-	Photosynthesis						
Chemical:	Di-ethylhexy	vl phthalate (DEHP)						
HERO ID:	3350318							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health. The experiments were conducted in an artificial climate chamber. The seedlings were cultured under a cycle of 12-h days at 25C and 12-h nights at 20C at 60% relative humidity level.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies for chlorophyll content, photosynthetic parame- ters, and chlorophyll fluorescence were reported in detail.				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.				
Domain 6: Confounding	y / Variable Cou	atrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported, but these are unlikely to have a substantial impact on results. Experi- ments were conducted in a controlled environment.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but calculations and measures of significance were not provided, so no conclusions about a dose response could be made. Results of multi- ple comparisons were not provided.				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	The author's conclusions	The author's discussion of the effects of DBP and DEHP on growth indices and mechanistic endpoints of wheat seedlings was not clear, and their conclusions were made without incorporating any discussion of statistical significance. Results of multiple comparisons were not provided.						

Overall Quality Determination

High

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Study Citation:	Ma, T. T., Cl	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil.						
Demotions	Pedosphere 2	Pedosphere 24(1):107-115.						
Duration: Exposure Route	Terrestrial: S	Terrestrial: Soil: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media. Path:	Terresultar, c	referenced by study authors (i.e., enclinear of interest in exposure water, but anable to determine exact uptake route)						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Vigna radiata; Not Applica	ble (e.g., fungi o	r algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2510954							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		Ŧ					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only				
	Metric 2:	Test Substance Source	LOW	The test substance identify was not analytically verified by the performing laboratory				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%				
Domain 2: Test Design								
C C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups				
Domain 3: Exposure Ch	aracterization							
2 oniuni et 2npooure en	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in				
		Preparation		adequate detail				
	Metric 8:	Consistency of Exposure	Medium	exposures were administered consistently across study groups, albeit with few details				
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type				
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose				
		Spacing of Exposure Levels		response				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.				
Domain 4: Test Organis	m							
8	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized				
	Metric 15	Conditions Number of Organisms and	Medium	The numbers of test organisms and realizates were reported and sufficient to character				
	Wieure 15.	Replicates per Group	Weddulli	ize toxicological effects				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest				
Continued on next page								

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Diethylhexyl Phthalate

HERO ID: 2510954 Table: 1 of 3

		contin	ued from previ	ous page				
Study Citation:	Ma, T. T., Cl	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil.						
	Pedosphere	Pedosphere 24(1):107-115.						
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)							
Exposure Route,	Terrestrial; S	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; Vigna radiata; Not Applicat	ole (e.g., fungi o	r algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2510954							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups				
		Assessment						
Domain 6: Confounding	y / Variable Co	ntrol						
Domain of Comountaing	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions				
		Design and Procedures	8	»				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.				
Domain 7: Data Present	ation and Anal	veic						
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were adequately described				
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group				
	Wieure 22.	Reporting of Data	Low	but results were described in the text.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes				
Additional Comments:	Seedling roo length was d	eedling root and shoot lengths were measured with a millimeter ruler, and the biomass (fresh weight, FW) in each dish was determined by weighing. Root ength was defined as the length from root tip to root radicle.						

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil. Pedosphere 24(1):107-115. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Di-ethylhexyl phthalate (DEHP) 2510054						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		1				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.6%.			
Domain 2. Test Design							
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		TT' 1				
	Metric /:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in adequate detail			
	Metric 8:	Consistency of Exposure	Medium	Exposures were administered consistently across study groups, albeit with few details.			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose			
	Wieute 11.	Spacing of Exposure Levels	Ingn	response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.			
Domain 4: Test Organis	m						
Domain 1. 10st Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
Domain 5: Outcome Ass	sessment						
2 small 5. Outcome Ast	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.			

Diethylhexyl Phthalate

HERO ID: 2510954 Table: 2 of 3

		contin	ued from previ	ous page			
Study Citation:	Ma, T. T., Cl	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil.					
	Pedosphere	24(1):107-115.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	1: 0 - 4 days (0-9	6h)			
Exposure Route,	Terrestrial; S	Soil; Not determined by study authors (i.e., c	hemical of inter	est in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Vigna radiata; Not Applical	ble (e.g., fungi o	r algae studies) or Not Reported			
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Di-ethylhexy	yl phthalate (DEHP)					
HERO ID:	2510954	2510954					
Domain	Metric Rating Comments						
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was nothing reported to indicate there were differences among groups that could influence the outcome assessment.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form is	to assess the germination outcome.					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in so Pedosphere 24(1):107-115. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)								
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V Mechanistic- Di-ethylhexy 2510954	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect) Di-ethylhexyl phthalate (DEHP) 2510954							
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only					
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory					
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%					
Domain 2: Test Design									
Domain 21 Test Dosign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group					
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported and adequate					
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups					
Domain 3: Exposure Ch	aracterization								
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail					
	Metric 8:	Consistency of Exposure	Medium	exposures were administered consistently across study groups, albeit with few details					
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured					
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type					
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose					
		Spacing of Exposure Levels	0	response					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.					
Domain 4: Test Organis	m								
-	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source					
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized					
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects					
Domain 5: Outcome Ass	sessment								
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health					
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	High High	The outcome assessment methodology reported the intended outcome of interest outcomes were assessed consistently across study groups					

Diethylhexyl Phthalate

HERO ID: 2510954 Table: 3 of 3

		conti	nued from p	previous page				
Study Citation:	Ma, T. T., Cl	Ma, T. T., Christie, P., Luo, Y. M., Teng, Y. (2014). Physiological and antioxidant responses of germinating mung bean seedlings to phthalate esters in soil.						
	Pedosphere 2	Pedosphere 24(1):107-115.						
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; S	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:								
Taxa, Species, Age:	Vegetation; '	Vascular Plants; Vigna radiata; Not Applica	able (e.g., fu	ngi or algae studies) or Not Reported				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)						
Chemical:	Di-ethylhexy	yl phthalate (DEHP)						
HERO ID:	2510954							
Domain		Metric	Rating	Comments				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described				
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes				
Additional Comments:	Mechanistic endpoints are: malondialdehyde (MDA) Polyphenol oxidase (PPO)ascorbate peroxidase (APX)Superoxide dismutase (SOF)The glutathione (GSH)peroxidase (POD)Proline content							
Overall Qualit	ty Deterr	nination	High					

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