

May 2025 Office of Chemical Safety and Pollution Prevention

Data Quality Evaluation Information for Environmental Hazard for Dibutyl Phthalate (DBP) (1,2-Benzenedicarboxylic acid, 1,2-dibutyl ester)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 84-74-2



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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 Dibutyl Phthalate (DBP)*. 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 performs data quality evaluation as a part of the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (referred to hereafter as the '2021 Draft Systematic Review Protocol'). The systematic review steps are further described in the *Draft Risk Evaluation for Dibutyl Phthalate (DBP) – Systematic Review Protocol*.

Different data quality evaluation forms were used depending on the organism as described in the PECO (Population, Exposure, Comparator or Scenario, and Outcomes) 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 table of contents lists references based on chemical (target chemical followed by analogue chemical), and 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.

Habitat: Aquatic (freshwater)

Taxa: Vertebrates

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676322		Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.	63
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788294		Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environmental Toxicology and Chemistry 30(6):1338-1345.	69
	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.	75
1316201		Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus).	77
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.	79
	Leuciscus idus L.		
10817969		Aktiengesellschaft,, BASF (1989). Report on the study of the acute toxicity of dibutylphthalat on the Golden Orfe (Leuciscus idus L., golden variety).	81
	Melanotaenia fluviatilis		
1639196		Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.	85
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6571362		EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.	113
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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.	119

Oncorhynchus mykiss (Salmo gairdneri)

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	Oreochromis niloticus		
3974179		Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685.	125
3350208		Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.	135
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1316189		Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions.	223

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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.	239
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	Salmo salar		
1332592		Tollefsen, K. E., Meys, J. F., Frydenlund, J., Stenersen, J. (2002). Environmental estrogens interact with and modulate the properties of plasma sex steroid-binding proteins in juvenile Atlantic salmon (Salmo salar). Marine Environmental Research 54(3-5):697-701.	249
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Taxa: Invertebrates

Brachionus calyciflorus

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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.	297
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	299
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.	303
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	305
	Chironomus tentans		
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.	307
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.	311
7325945		Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.	315
	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.	323
1316223		Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to Daphnia magna (final report).	325
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.	328
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.	330
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1316195	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to Daphnia magna with cover letter dated 032585. :95.	382
Gamma	us pseudolimnaeus	
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Gamma	us pulex	
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.	390
Hexager	ia 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.	396
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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.	398
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7325945		Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.	404
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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.	418
	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.	420
7325945		Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.	422
	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.	424
	Orconectus nais		
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.	430
	Paleomonetes kadiakensis		
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.	432
	Paratanytarsus parthenogen	etica	
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.	434
	Paratanytarsus parthenogen	ica	
1316219		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424.	436
Т	Caxa: Plants (Non-vascul	lar)	
	Algae		
1332820		Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-608.	438
	Chlorella emersonii		
1333016		Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.	440

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	Chlorella pyrenoidosa		
5433509		Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130.	444
	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.	448
	Pseudokirchneriella subcapi	itata	
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.	450
	Scenedesmus obliquus		
5433509		Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130.	452
1332820		Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-608.	456
1332820		Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-608.	458
	Selenastrium capricornutum		
1316196		Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga Selenastrum capricor- nutum.	462
	Selenastrum capricornutum		
1323217		Adachi, A., Asa, K., Okano, T. (2006). Efficiency of rice bran for removal of di-n-butyl phthalate and its effect on the growth inhibition of Selenastrum capricornutum by di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 76(5):877-882.	464
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.	468
1333016		Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.	470
Т	Caxa: Plants (Vascular)		
	Hordeum vulgare		
1333016		Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.	472

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1323213		Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626.	474
	Spinacea oleraceae		
1333016		Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.	478
	Spirodela polyrhiza		
1323213		Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626.	480
	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.	484
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.	486
Т	Taxa: Other		
	fungus		
1323196	,	Liang, W., Deng, J. Q., Zhan, F. C., Wu, Z. B. (2009). Effects of constructed wetland system on the removal of dibutyl phthalate (DBP). Microbiological Research 164(2):206-211.	492
Hał	oitat: Aquatic (mar	ine)	
]	Taxa: Vertebrates		
	sheepshead minnow (Cypri	inodon variegatus)	
1316224		Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report).	494
]	axa: Invertebrates		
	Animalia		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	496
	Annelida		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	500
	Artemia salina		

Dibutyl Phthalate		Table of Contents	
5569571		Sugawara, N. (1974). Effect of phthalate esters on shrimp. Bulletin of Environmental Contamination and Toxicology 12(4):421-424.	504
	Arthropoda		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	508
	Chordata		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	512
	Coelenterata		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	516
	Echinodermata		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	520
	Haliotis diversicolor super	texta	
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.	524
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.	526
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.	528
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.	534
	Mollusca		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	536
	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.	540
1316220		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).	542

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Dibutyl P	Phthalate	Table of Contents	
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.	544
	Palaemonetes pugio		
5557723		Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408.	550
5557723		Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408.	552
	Rhynchocoela		
5495608		Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	554
Т	'axa: Plants (Non-vascu	lar)	
	Dunaliella parva		
790153		Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.	556
	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.	558
	Skeletonema costatum		
789981		Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environ- mental Contamination and Toxicology 25(1):75-78.	564
	Synechococcus lividus		
790153		Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.	572
	Thalassioria pseudomona		
790153		Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.	574
Hab	oitat: Aquatic (brac	kish)	
Т	faxa: Vertebrates		

Cyprinodon variegatus

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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.	576
	sheepshead minnow (Cyp	rinodon variegatus)	
1316224		Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report).	578
	Xenopus laevis		
128004		Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.	580
]	Taxa: 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.	588
	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.	590
	Mysidopsis bahia		
1316220		Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).	592
	Nitocra spinipes		
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.	594
	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.	596
Hal	oitat: Terrestrial		
r	Taxa: Vertebrates		
	Capra hircus		
1332948		Cornell University, (1931). Report upon the toxicity of plasticizers.	598
	Coturnix coturnix		

Dibutyl F	hthalate	Table of Contents	
2346127		Bello, U. M., Madekurozwa, M.,-C, Groenewald, H. B., Aire, T. A., Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Compar- ative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 166:24-33.	612
	Gallus domesticus		
1332945		DuPont, (1949). Toxicity of dibutyl phthalate.	618
	Gallus gallus		
1332948		Cornell University, (1931). Report upon the toxicity of plasticizers.	622
1332948		Cornell University, (1931). Report upon the toxicity of plasticizers.	632
	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.	648
	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.	654
Т	axa: Invertebrates		
	Caenorhabditis elegans		
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.	656
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.	664
	Dermatophagoides farinae		
485854		Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.	672
1332803		Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Der- matophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861.	676
1323180		Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Experimental and Applied Acarology 44(1):1-9.	680
1341977		Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatophagoides farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031.	682

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788260		Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371.	684
	Dermatophagoides pteronys	ssinus	
485854		Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.	686
1332803		Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Der- matophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861.	690
1323180		Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Experimental and Applied Acarology 44(1):1-9.	694
1341977		Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatophagoides farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031.	696
788260		Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371.	698
	Drosophila melanogaster		
2510760		Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.	700
2510760		Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.	704
3350270		Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.	706
2510760		Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.	716
2510760		Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.	724
	Eisenia fetida		
2816887		Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669.	728
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.	730
2816887		Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669.	732

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2816887		Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669.	734
	Eutrombicula hirsti		
1341925		Frances, S. P. (1994). Response of a chigger, eutrombicula-hirsti (acari, trombiculidae) to repellent and toxicant compounds in the labora- tory. Journal of Medical Entomology 31(4):628-630.	736
	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.	740
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.	742
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.	746
	Lasius niger		
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.	750
	Meloidogyne incognita		
3350275		Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita. PLoS ONE 11(4):e0154675.	752
3350275		Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita. PLoS ONE 11(4):e0154675.	756
3350275		Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita. PLoS ONE 11(4):e0154675.	758
	Spodoptera frugiperda		
2219889		Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977.	760
	Tyrophagus putrescentiae		
1323221		Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoterpenoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557.	770
Т	axa: Plants (Vascular)		
	Achilla millefolium		
9430481		Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199.	778

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5551990		Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.	786
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.	788
	Brassica campestris		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	794
	Brassica napus		
4829418		Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150.	798
	Brassica oleracea		
5678863		Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.	804
	Brassica parachinensis		
5043543		Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551.	816
	Brassica parachinensis L.		
3070947		Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849.	824
	Brassica rapa		
1296241		Liao, C. S., Yen, J. H., Wang, Y. S. (2009). Growth inhibition in Chinese cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate. Journal of Hazardous Materials 163(2-3):625-631.	836
1298079		Liao, C. S., Yen, J. H., Wang, Y. S. (2006). Effects of endocrine disruptor di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis). Chemosphere 65(10):1715-1722.	842
5605728		Zhu, F., Zhu, C., Chen, N., Zhou, D., Gao, J. (2018). Will spent mushroom substrate application affect the dissipation and plant uptake of phthalate esters?. Journal of Soils and Sediments 18(4):1579-1589.	848
	Browallia speciosa		
1333234		Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-163.	850
	Carica papaya		

Dibutyl F	hthalate	Table of Contents	
5433168		Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis recovery. Journal of Plant Nutrition 10(9-16):1051-1058.	856
	Cucumis sativus		
5551990		Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.	862
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.	864
3502464		Wang, L., Sun, X., Chang, Q., Tao, Y., Wang, L., Dong, J., Lin, Y., Zhang, Y. (2016). Effect of di-n-butyl phthalate (DBP) on the fruit quality of cucumber and the health risk. Environmental Science and Pollution Research 23(23):24298-24304.	870
	Gossypium		
1639289		Wu, Y., Yuan, S. L. (2012). Dibutyl phthalate pollution on cotton growth and physiological characteristics of cotton. Advanced Materials Research 518-523:5436-5441.	874
	Holcus lanatus		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	878
	Leptochloa chinensis		
5432995		Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serrulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.	882
5432995		Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serrulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.	884
	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.	886
	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.	892
	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.	898
	Nicotinana tobacum		

Dibutyl I	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.	902
	Oryza sativa		
5551990		Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.	910
5432995		Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serrulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.	920
	Phaseolus vulgaris		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	922
	Picea abies		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	924
	Plantago major		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	928
	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.	930
1333234		Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-163.	936
	Sinapis Alba		
9430481		Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199.	942
	Sinapsis alba		
680337		Løkke, H., Bro-Rasmussen, F. (1981). Studies of mobility of di-iso-butyl phthalate (DiBP), di-N-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10(11-12):1223-1236.	944
	Sorghum bicolor		
5433174		Kannan, S. (1986). Effects of dibutyl phthalate and phthalic-acid on chlorosis recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant Nutrition 9(12):1543-1551.	948
	Trifolium repens		

Dibutyl P	hthalate	Table of Contents		
1302103		Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.	956	
	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.	960	
	Triticum aestivum L.			
5495646		Gao, M., Guo, Z., Dong, Y., Song, Z. (2019). Effects of di-n-butyl phthalate on photosynthetic performance and oxidative damage in different growth stages of wheat in cinnamon soils. Environmental Pollution 250:357-365.	966	
	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.	970	
	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.	974	
	Vigna sinensis			
5495799		Wang, S. G., Lin, X. G., Yin, R., Hou, Y. L. (2003). Effects of di-n-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants. Biologia Plantarum 47(4):637-639.	980	

Study Citation:	Ren, Z., Ren, B., Ren, B., Chen, B., Pan, H., Li, S., Xu, S., Tae-Soo, C., Wang, W. (2019). Is circadian rhythm a good indicator in the environmental assessment? The toxic effects of contaminants in trace level on the behavior responses of goldfish (Carassius auratus). Ecological Indicators 405:700-708.						
Duration: Exposure Route, Media, Path:	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:	Vertebrate; F	Fish; Carassius auratus; Adult					
Health Outcome:	Behavioral						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5673506						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name and molecular formula only.			
	Metric 2:	Test Substance Source	Low	The test substance was reported to be from Chinese Standard Sample Center, but it was not reported to be analytically verified.			
	Metric 3:	Test Substance Purity	High	The test substance was technical grade with $>95\%$ purity.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent solvent control in which 0.5% DMSO was used, which is equal to or greater than the concentrations of DMSO used in exposure groups.			
	Metric 5:	Negative Control Response	High	The solvent control response was reported in Figures 1-6 and was adequate.			
	Metric 6:	Randomized Allocation	Medium	Researchers reported one male and one female goldfish were randomly allocated to each test chamber.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	Little details were provided on the preparation of the test stock solution and test con- centration. Analytical verification of exposure concentrations was not reported. Vessel material was not reported.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for six days in a flow through system with test chambers that were 15cm long and 10cm in diameter at $22 + 2 C$.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substances were measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The duration was reported to be six days. This was adequate to see a response.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level as the goal was not to have a dose response, but to compare the responses of different chemicals			
	Metric 12:	Testing at or Below Solubility Limit	High	The DBP concentration was below the water solubility limit, and a vehicle solvent was used with an appropriate solvent control response.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The adult goldfish were from the Institute of Environment and Ecology at Shandong Normal University in China. Goldfish are a relevant species for this test.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Pretreatment environmental conditions were the same as test conditions. Acclimation to the flow-through cylindrical chambers prior to treatment was not reported.			
	Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 5673506 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation: Duration: Exposure Route, Modia Poth:	Ren, Z., Ren, B., Ren, B., Chen, B., Pan, H., Li, S., Xu, S., Tae-Soo, C., Wang, W. (2019). Is circadian rhythm a good indicator in the environmental assessment? The toxic effects of contaminants in trace level on the behavior responses of goldfish (Carassius auratus). Ecological Indicators 405:700-708. 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)						
Tava Species Age	Vertebrate: F	Fish: Carassius auratus: Adult					
Health Outcome	Rehavioral	ish, Curassias auraias, Maur					
Chemical:	Dibutyl phth	valate (DBP)					
HERO ID:	5673506						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were two goldfish per test chamber and four replicates per test chemical.			
Domain 5: Outcome As	sessment	al and her an all					
	Metric 16:	Adequacy of Test Conditions	Medium	The goldfish were kept at 22C with a 16L:8D photoperiod (4000 lux) in a test cham- ber containing two goldfish. The flow rate was maintained at 2L/h. The fish were fed Limnodrilus hoffmeisteri and flake fish food. pH and DO were not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-behavioral changes due to DBP 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: 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	vsis					
	Metric 21:	Statistical Methods	High	The statistical methods were described in detail in the "Data analysis" section of the paper.			
	Metric 22:	Reporting of Data	High	The data for the exposure and control responses were reported in Figures 1-6.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes.			
Additional Comments:	This evaluat	ion was on the effect of DBP on C. auratus c	circadian rhythm	s. The behavioral outcome was selected as the outcome of interest.			
Ovorall Queli	ty Dotorr	nination	Modium				
Viciali Quali	iy Deich		TATCATAILI				

Study Citation:	Pfuderer, P., depressors, F	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:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported						
Exposure Route, Modia Pathy	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	ish; Carassius auratus; Juvenile						
Health Outcome:	Cardiovascul	lar						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1333101							
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	High	The chemical purity was reported as 99.94%.				
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	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				
		Preparation		test concentrations. The cited reference for methods (Francis et al., 1975) was unavail- able, therefore this metric score reflects the amount of details provided in the study being reviewed.				
	Metric 8:	Consistency of Exposure Administration	Uninformative	A sonicated emulsion was used for exposure, presumably for all concentrations. Exposure administration cannot be expected to be consistent 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/	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	Low	An emulsion was used, so there is high probability at least one concentration exceeds				
	metrie 12.	Testing at of Below Solution y Emile	2011	solubility.				
Domain 4: Test Organie								
Domain 4. Test Organis	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.				
			Continued on next page					

Environmental Hazard Evaluation

HERO ID: 1333101 Table: 1 of 1

		сог	ntinued from previous	page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	y Citation: 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. tion: Overall Duration: Not-reported; Exposure Duration: Not-reported sure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) ia, Path: , Species, Age: Vertebrate; Fish; <i>Carassius auratus</i> ; Juvenile th Outcome: Cardiovascular Dibutyl phthalate (DBP) O U: 1232101						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates was not reported. The figure on heart rate activity (Fig 6) showed only the response of an individual fish but mean heart rate was given in Table 1. The cited reference for methods (Francis et al., 1975) was un-available, therefore this metric score reflects the amount of details provided in the study being reviewed.			
Domain 5: Outcome A	ssessment						
	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 re- viewed.			
Domain 6: Confoundir	og / Variable Co	ntrol					
Domain of Comoundin	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 Preser	ntation and Anal	vsis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High High	Data for exposure-related findings were presented for each treatment group (Table 1). There were no unexpected outcomes. Heart rate data was provided with some measure of variability in Table 1.			
Additional Comments:	Overall, a po exposure con was provider	borly designed and poorly written study. A non- ncentrations, duration of exposure were not p d for DBP for the concentrations tested in Tab	egative control group w rovided. It was reported le 1. The cited reference	as not reported. Details of experimental system, test media preparation, I that a sonicated emulsion was used for exposure. Mean heart rate data e for methods was not available at the time of review.			

Overall Quality Determination

Dibutyl Phthalate

Uninformative

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						
Duration: Exposure Route, Media. Path:	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:	Age: Vertebrate: Fish: Cyprinodon variegatus: Juvenile						
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature reported without CASRN			
	Metric 2:	Test Substance Source	Low	Reported as provided by manufacturer from commercially available batches. Manufac- ture name and batch number not provided. No analytical data reported.			
	Metric 3:	Test Substance Purity	High	At least 95% purity			
Domain 2: Test Design							
C C	Metric 4:	Negative Controls	High	Negative control reported			
	Metric 5:	Negative Control Response	High	Control response acceptable			
	Metric 6:	Randomized Allocation	Low	Allocation method not reported.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Medium	Experimental system well described. However, headspace or measures to prevent			
	Metico	Preparation	TI: 1	volatilization not repetid.			
	Metric 8:	Consistency of Exposure	High	Exposure administration consistent across groups.			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at start and 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 vessels.			
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure appropriate for test.			
	Metric 11:	Number of Exposure Groups/	High	Exposure levels appropriate. Range finding test was performed.			
	Metric 12:	Testing at or Below Solubility Limit	High	Test performed at or below water solubility			
Domain 4: Test Organis	m						
c	Metric 13:	Test Organism Characteristics	Low	Source not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for test 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	Environmental conditions appropriate for test.			
		Cont	inued on nex	xt page			

Environmental Hazard Evaluation

HERO ID: 1321996 Table: 1 of 1

continued from previous 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						
Destin	organisms. H	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96n); Exposure Duratio	n: 0 - 4 days					
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:	X7 (1 (T							
Taxa, Species, Age:	vertebrate; F	fish; Cyprinodon variegatus; Juvenile						
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1321996							
Domain		Metric	Rating	Comments				
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.				
	Metric 18:	Consistency of Outcome	High	Outcome assessment consistent across groups.				
		Assessment						
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.				
		Design and Procedures	-					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences between groups.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Statistical methods performed and described.				
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints reported.				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.				
Additional Comments:	None							
Overall Qualit	Overall Ouality Determination H							

······································	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of								
Duration: Exposure Route, Madia Pathy	Cyprinus car 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:	Vertebrate: F	Fish: Cyprinus carnio: Juyenile							
Health Outcome:	Respiratory								
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	3071043								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce								
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.					
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.					
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 100% pure.					
Domain 2: Test Design									
-	Metric 4:	Negative Controls	High	Study authors reported using a solvent control.					
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and Figure 1 and was adequate for the outcomes of interest.					
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.					
Domain 3: Exposure Ch	aracterization								
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Low	Few details were provided on the preparation of test substance. Vessel material was not reported.					
	Metric 8:	Consistency of Exposure Administration	Low	All tests were conducted in 120L tanks, but little other information was provided on test consistency.					
	Metric 9:	Measurement of Test Substance	Low	It was not reported if test concentrations were measured at any point.					
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 96h with samples taken at 4, 24, and 96h. This was adequate to observe a response.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	3 exposure groups $(1, 5, and 10 \text{ mg/L})$ were tested in a range-finder to determine an appropriate sublethal dose. These were appropriate concentrations for the range-finder, as a sublethal dose was identified as 1 mg/L. This is marked as N/A, because the authors' intention for the study was to assess effects from a sublethal concentration over a time course.					
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the solubility limit, and acetone was used as a vehicle solvent.					
Domain 4: Test Organis	m								
<u>.</u>	Metric 13:	Test Organism Characteristics	Medium	The carp are an appropriate test species and were reported to be juveniles. They were not obtained from a reliable source, they were obtained from the State Hydraulic Works General Directorate, DSI, Yedikir Dam Lake, Amasya, Turkey, indicating they were wild-caught.					

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Dibutyl Phthalate

		contin	ued from previ	ious page		
Study Citation: Duration:	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of Cyprinus carpio. Molecular Biology Reports 42(9):1409-1417.					
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; I	Fish; Cyprinus carpio; Juvenile				
Health Outcome:	Respiratory					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3071043					
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment	High	The carp were acclimated for 15d prior to the start of the study.		
	Matria 15.	Conditions	Law			
	Methe 15.	Replicates per Group	LOW	were in the control group, but the number of replicates (fish tanks) was not reported		
		Replicates per Gloup				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept in 120L tanks with a 12L:12D photoperiod in dechlorinated tap water. It was reported the fish were fed commercial fish food and were then starved prior to the study. The feeding regimen was not reported. More information is needed on temperature as well.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–Histopathological effects on the gills.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. At each sample period, fish tissue was fixed and later examined using light microscopy.		
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. Study authors needed to provide more information on the environmental conditions of the fish. Water quality measurements are reported, but only one number for each parameter is given, making it unclear if these are representative of control or exposure group conditions, or an average of both, or whether conditions differed between control and exposure 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 methods were described in the "Data Analysis" section of the paper and were appropriate.		
	Metric 22:	Reporting of Data	High	Results were reported in Table 1 and Figures 1 and 2 for the control response and the exposure response. The data was appropriate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion	of the study was on the histopathological ef	ffects of DBP on	C. carpio gills. The respiratory outcome was selected as the outcome of interest.		

Overall Quality Determination

Medium

Study Citation:	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of								
Duration	Cyprinus car	pio. Molecular Biology Reports 42(9):1409-	-1417.	(h)					
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 untake route)							
Media. Path:	require (neshwater), water, not determined by study autions (ne., enemiear of merest in exposure water, but anable to determine exact aptake route)								
Taxa, Species, Age:	Vertebrate; F	ish; Cyprinus carpio; Juvenile							
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)							
Chemical:	Dibutyl phtha	alate (DBP)							
HERO ID:	3071043								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	Ce Matria 1.	Test Substance Identity	Iliah						
	Metric 1: Metric 2:	Test Substance Source	Low	The DBP was identified by CASKN.					
	Meuric 2.	Test Substance Source	LOW	fied.					
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 100% pure.					
Domain 2: Test Design									
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a solvent control.					
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 5 and 6 and was adequate for the outcomes of interest.					
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.					
Domain 3: Exposure Ch	aracterization								
	Metric 7:	Experimental System/Test Media Preparation	Low	Few details were provided on the preparation of the test substance. Vessel material was not reported.					
	Metric 8:	Consistency of Exposure	Low	All tests were conducted in 120L tanks, but little other information was provided on test					
	Metric 9:	Measurement of Test Substance	Low	It was not reported if test concentrations were measured at any point.					
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 96h with samples taken at 4, 24, and 96h. This was adequate to observe a response.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Three exposure groups (1, 5, and 10 mg/L) were tested in a range-finder to determine an appropriate sublethal dose. These were appropriate concentrations for the range-finder, as a sublethal dose was identified as 1 mg/L. This metric is marked as N/A, because the authors' intention for the study was to assess effects from a sublethal concentration over a time course.					
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the solubility limit, and acetone was used as a vehicle solvent.					
Domain 4: Test Organisi	n								
	Metric 13:	Test Organism Characteristics	Medium	The carp are an appropriate test species and were reported to be juveniles. They were not obtained from a reliable source, they were obtained from the State Hydraulic Works General Directorate, DSI, Yedikir Dam Lake, Amasya, Turkey, indicating they were wild-caught.					
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The carp were acclimated for 15d prior to the start of the study.					
		Contin	ued on next pa	ge					

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Environmental Hazard Evaluation

HERO ID: 3071043 Table: 2 of 3

Study Citation:	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of						
Duration:	Cyprinus carpio. Molecular Biology Reports 42(9):1409-1417. 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) Vertebrate; Fish; <i>Cyprinus carpio</i> ; Juvenile						
Media, Path:							
Taxa, Species, Age:							
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	3071043						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Five to seven organisms were sampled per time point in the treatment group and 16 organisms were in the control group, but the number of replicates (fish tanks) was not reported.			
Domain 5: Outcome A	ssessment						
2	Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept in 120L tanks with a 12L:12D photoperiod in dechlorinated tap water. It was reported the fish were fed commercial fish food and were then starved prio to the study. The feeding regimen was not reported. More information is needed on temperature as well.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in HSP70 expression over time.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Real time PCR was used to measure HSP70 in the gill and the liver, and Western Blot was used to measure HSP70 protein levels.			
Domain & Confoundin	a / Variabla Ca	ntrol					
Domani O. Comoundin	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions. Study authors needed to provide more information on the environmental			
				conditions of the fish. Water quality measurements are reported, but only one number for each parameter is given, making it unclear if these are representative of control or ex posure group conditions, or an average of both, or whether conditions differed between control and exposure 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	lycic					
2 omain /	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate.			
	Metric 22:	Reporting of Data	High	Results were reported in Figures 5 and 6 for the exposure response and the control re- sponse. Data presented was appropriate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures.			
Additional Comments:	This portion outcome of i	a of the study was on the effect of DBP on interest.	HSP70 express	ion and protein levels. The mechanistic biomarker outcome was selected as the			

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Dibutyl Phthalate

HERO ID: 3071043 Table: 2 of 3

		continued from previous page				
Study Citation:	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dir	nçel, A., Erkoç, F. (2015). Impact of I	OBP on histology and expression of HSP 70 in gill and liver tissue of			
	Cyprinus carpio. Molecular Biology Reports 42	2(9):1409-1417.				
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; Cyprinus carpio; Juvenile					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	3071043					
Domain	Matria	Dating	Comments			

Domain	Metric	Rating	Comments
Overall Quality De	etermination	Medium	

Study Citation:	Agus, H. H.,	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of						
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	ish; Cyprinus carpio; Juvenile						
Health Outcome:	Hepatic/Live	r						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	3071043							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		TT 1					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.				
	Metric 2:	lest Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.				
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 100% pure.				
Domain 2: Test Design								
Domani 2. Test Design	Metric 4.	Negative Controls	High	Study authors reported using a solvent control				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and Figure 3 and was adequate				
		Sector Commences Former	8	for the outcomes of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.				
	, . <i>.</i> .							
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Law					
	Metric 7:	Preparation	Low	rew details were provided on the preparation of test substance. Vessel material was not reported.				
	Metric 8:	Consistency of Exposure	Low	All tests were conducted in 120L tanks, but little other information was provided on test				
	Matria 0:	Administration	Low	consistency.				
	Mettic 9.	Concentration	Low	It was not reported if test concentrations were measured at any point.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h with samples taken at 4, 24, and 96h. This was adequate to observe a response.				
	Metric 11:	Number of Exposure Groups/	N/A	3 exposure groups (1, 5, and 10 mg/L) were tested in a range-finder to determine an ap-				
		Spacing of Exposure Levels		a sublethal dose was identified as 1 mg/L. This is marked as N/A, because the authors' intention for the study was to assess effects from a sublethal concentration over a time course.				
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the solubility limit, and acetone was used as a vehicle solvent.				
Domain 4: Test Organist	n							
	Metric 13:	Test Organism Characteristics	Medium	The carp are an appropriate test species and were reported to be juveniles. They were not obtained from a reliable source, they were obtained from the State Hydraulic Works General Directorate, DSI, Yedikir Dam Lake, Amasya, Turkey, indicating they were wild-caught.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The carp were acclimated for 15d prior to the start of the study.				
		Contin	ued on next pa	ge				

Environmental Hazard Evaluation

HERO ID: 3071043 Table: 3 of 3

		contin	ued from previ	ious page			
Study Citation: Duration: Exposure Route, Media. Path:	Agus, H. H., Erkmen, B., Sümer, S., Sepici-Dinçel, A., Erkoç, F. (2015). Impact of DBP on histology and expression of HSP 70 in gill and liver tissue of Cyprinus carpio. Molecular Biology Reports 42(9):1409-1417. 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 Hepatic/Live Dibutyl phth 3071043	Fish; <i>Cyprinus carpio</i> ; Juvenile er lalate (DBP)					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 to 7 organisms were sampled per time point in the treatment group and 16 organisms were in the control group, but the number of replicates (fish tanks) was not reported.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept in 120L tanks with a 12L:12D photoperiod in dechlorinated tap water. It was reported the fish were fed commercial fish food and were then starved prior to the study. The feeding regimen was not reported. More information on temperature is needed.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–Histopathological effects on the liver.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. At each sample period, fish tissue was fixed and later examined using light microscopy.			
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. Study authors needed to provide more information on the environmental conditions of the fish. Water quality measurements are reported, but only one number for each parameter is given, making it unclear if these are representative of control or exposure group conditions, or an average of both, or whether conditions differed between control and exposure 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	veie					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate.			
	Metric 22:	Reporting of Data	High	Results were reported in Table 1 and Figures 3 and 4 for the control response and the exposure response. The data was appropriate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			

Overall Quality Determination

Medium

Study Citation:	Poopal, R. K., Ramesh, M., Maruthappan, V., Rajendran, R. B. (2017). Potential effects of low molecular weight phthalate esters (C16H22O4 and C12H14O4) on the freshwater fish Cyprinus carpio. Toxicology Research 6(4):505-520.						
Duration: Exposure Route, Media Path	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>Cyprinus carpio</i> ; Juvenile						
Taxa. Species. Age:							
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3974208						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be from Loba Chemie Pvt. Ldt. in Mumbai, India. It was reported the DBP did not have any impurities. The test substance identity was NOT analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	It was reported the DBP was analytical grade and 99.99% pure.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	It was reported a toxicant free negative control group was used.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Low	Little information on the preparation of the test concentrations was reported. The system was reported to be a static system. Experiments were conducted in glass tanks.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 96h in 20L glass tanks with 10 fish per tank and three replicates per concentration. Fish were monitored daily for mortalities.			
	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:	Concentration Exposure Duration and Frequency	High	The exposure duration was 96h, which is typical of a fish acute toxicity test.			
	Metric 11:	Number of Exposure Groups/	High	There were seven exposure levels, and the spacing was adequate to obtain LC50 values.			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	Five out of seven concentrations of the DBP tested were above the water solubility limit. Study authors did not report if the negative control was a solvent control. According to the scope document, the water solubility of DBP is 11.2 mg/L at 25° C. DBP concentrations tested were 5, 10, 15, 20, 25, 35 and 40 mg L-1.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from the Tamil Nadu Fisheries Development Corporation in Aliyar, Tamil Nadu, India. Fingerlings were reported to be used in the study.			
	Metric 14:	Acclimatization and Pretreatment	High	The fish were acclimated to lab conditions for 30 days prior to testing.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were ten fish per test chamber with three replicates per concentration.			
			Continued on next page				

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 3974208 Table: 1 of 1

		0	ontinued from previous	page				
Study Citation:	Poopal, R. I C12H14O4) Overall Dur	Poopal, R. K., Ramesh, M., Maruthappan, V., Rajendran, R. B. (2017). Potential effects of low molecular weight phthalate esters (C16H22O4 and C12H14O4) on the freshwater fish Cyprinus carpio. Toxicology Research 6(4):505-520.						
Exposure Route, Media, Path:	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; I	Sish; Cyprinus carpio; Juvenile						
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	3974208							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
Domain 5. Outcome ris	Metric 16:	Adequacy of Test Conditions	Medium	The fish were kept in 20L tanks during the study. Prior to the study they were fed rice bran and ground nut oil cake dough ad libitum. It was reported the they were kept at natural temperatures and photoperiod, but it was not specified what these were.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–LC50 values.				
	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 observed every 24h for mortality, and dead fish were removed immediately.				
Domain 6: Confounding	y / Variable Co	atrol						
	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	LC50 values were determined by probit analysis.				
	Metric 22:	Reporting of Data	Low	Only LC50 values were reported in the text. Control and exposure responses were not reported.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. The 95% confidence intervals were not reported.				
Additional Comments:	: This portion of the evaluation is on the 96h acute toxicity of DBP. Mortality was selected as the outcome of interest. The study received an unacceptable rating due to the fact that five out of seven concentrations tested were above the DBP water solubility limit, and it was not reported if the negative control contained a vehicle solvent.							
Overall Quali	ty Detern	nination	Uninformativ	e				
Study Citation:	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							
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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:	Vertebrate; Fish; Cyprinus carpio; Adult							
Health Outcome:	Mortality	alata (DDD)						
HERO ID:	2510817	alate (DDF)						
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 Fullty	nigii	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	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							
Ĩ	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.				
	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.				
	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 16.3 mg/L for DBP. The solubility listed in the Final Scope for this compound is 2.69 mg/L.				
Domain 4. Test Organis	m							
2 chian ii rost organis	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the choice of source of 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	Low	The number of test organisms and replicates were lower than the typical number.				
		Repletes 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 ade- quate.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
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HERO ID: 2510817 Table: 1 of 1

		····contina	aca nom prem	ous page			
Study Citation:	Zhao, X., Ga 23(4):626-62	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:	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:	Vertebrate; H	Fish; Cyprinus carpio; Adult					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
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.			
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	C C				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
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	Hıgh	There were no unexpected outcomes.			
Additional Comments:	The reported LC50 from this study was 16.3 mg/L for DBP. The solubility listed in the Final Scope for this compound is 2.69 mg/L.						
Overall Quality Determination Medium							

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

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: Exposure Route, Media, Path:	23(4):626-63 Overall Dura Aquatic (free	Diverall 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:	Vertebrate; F	ish; Cyprinus carpio; Adult					
Health Outcome:	Mechanistic-	Mechanistic-Biomarkers (exposure and effect)					
HERO ID:	2510817	alate (DBP)					
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	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 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	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.			
	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 a dose response.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Two of the three treatment concentrations (3.26 and 8.15 mg/L) are above the solubility listed in the final scope for DBP at 2.6 mg/L.			
Domain 4: Test Organis	m						
0	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the choice of source of 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	Low	The number of test organisms and replicates were lower than the typical number.			
		Repleates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently reported to evaluate if ade- quate.			
	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

continued from previous page					
Study Citation:	Zhao, X., Ga	ao, Y., Qi, M. (2014). Toxicity of phthalate	esters exposure to	o carp (Cyprinus carpio) and antioxidant response by biomarker. Ecotoxicology	
	23(4):626-63	32.			
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:	Vertebrate; Fish; Cyprinus carpio; Adult				
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)			
Chemical:	Dibutyl phth	alate (DBP)			
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.	
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	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 Medium					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

			D D (201)			
Study Citation:	Poopal, R. I C12H14O4)	C12H14O4) on the freshwater fish Cyprinus carpio. Toxicology Research 6(4):505-520.				
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; I	Aish; Cyprinus carpio; Juvenile	1. 10			
Health Outcome:	Mechanistic-	-Biomarkers (exposure and effect)-Cell sign	aling/function-C	Didative stress (including redox biology)-Immune/Hematological-Nutritional and		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3974208					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be from Loba Chemie Pvt. Ldt. in Mumbai, India. It was reported the DBP did not have any impurities. The test substance identity was NOT analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	It was reported the DBP was analytical grade and 99.99% pure.		
Demain 2. Test Design						
Domain 2: Test Design	Metric 4:	Negative Controls	High	It was reported a toxicant free negative control group was used as well as a control con- taining acetone reported as the positive control.		
	Metric 5:	Negative Control Response	Low	The biological responses of the negative control and the solvent control were reported in Figures 1-12 and in Table 1 and were adequate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.		
	, · ,·					
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	Little information on the propagation of the test concentrations uses reported. The system		
	Methe 7.	Preparation	Low	was reported to be a static renewal treatment with daily water renewals to remove waste and renew the toxicant.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 35 days in 100L glass aquaria with 50 fish each. There were daily renewals and samples taken at days 7, 14, 21, 28, and 35.		
	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:	Concentration Exposure Duration and Frequency	High	The exposure duration was 35 days. This was adequate for a response to be observed		
	Metric 11:	Number of Exposure Groups/	Low	There were only two exposure levels, which is lower than is typical, but adequate for the		
		Spacing of Exposure Levels		outcomes of interest.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The study authors reported the use of a positive control (acetone) and a negative control (no acetone or toxicant) in the results section but the test solution preparation details were not mentioned. The solvent concentration was not reported.		
Domain 4: Test Organia	m					
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	High	The fish were reported to be from the Tamil Nadu Ficheries Development Corporation in		
	mente 15.	Test Organism Characteristics	ingn	Aliyar, Tamil Nadu, India. Fingerlings were reported to be used in the study.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were acclimated to lab conditions for 30 days prior to testing.		
		Contin	nued on next pa	age		

HERO ID: 3974208 Table: 1 of 1

		contir	nued from previ	ious page			
Study Citation:	Poopal, R. I	Poopal, R. K., Ramesh, M., Maruthappan, V., Rajendran, R. B. (2017). Potential effects of low molecular weight phthalate esters (C16H22O4 and C12H14O4) on the freshwater fish Cyprinus carrie. Toxicology Research 6(4):505-520					
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	davs	n 0(1).505 520.			
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; Cyprinus carpio; Juvenile					
Health Outcome:	Mechanistic-	-Biomarkers (exposure and effect)-Cell sign	aling/function-C	Oxidative stress (including redox biology)-Immune/Hematological-Nutritional and			
~	Metabolic						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3974208						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 50 fish per test chamber. Study authors reported a "suitable" number of replicates were used, but did not specify what these numbers were.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The fish were kept in 100L tanks during the study. Prior to the study they were fed rice bran and ground nut oil cake dough ad libitum. It was reported that they were kept at natural temperatures and photoperiod, but it was not specified what these were.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-a number of mechanistic outcomes.			
	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 to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	The study author described the statistical methods in the "statistical analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the control and exposure responses were reported in Table 2 and in Figures 1- 12 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 is on the effect of DBP on several mechanistic outcomes.						

Overall Quality Determination

Medium

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				
Duration: Exposure Route, Media Path:	phthalates an Overall Dura Aquatic (free	ad their mixtures. International Journal of E 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	search and Public Health 11(3):3156-3168. 6h) nical of interest in exposure water, but unable to determine exact uptake route)	
Taxa. Species. Age:	Vertebrate: F	Fish: Danio rerio: AB Strain: Embryo			
Health Outcome:	Mortality	,,,,,			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	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 are reported.	
	Metric 2:	Test Substance Source	High	Sources were listed.	
	Metric 3:	Test Substance Purity	Low	Purity/grade were not reported.	
Domain 2: Test Design					
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 Preparation	Low	Dilution of the test substance into medium was not well described (unclear if embryo rearing medium was utilized) and the test substance was not renewed over 72 hr.	
	Metric 8:	Consistency of Exposure	Medium	Exposures appear to have been administered consistently.	
	Metric 9:	Measurement of Test Substance	Low	Concentrations are reported as nominal.	
	Metric 10:	Exposure Duration and Frequency	Medium	Acute exposures were 72-hr for 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, 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 Organism					
8	Metric 13:	Test Organism Characteristics	High	Source and strain were reported and husbandry methods were adequately described.	
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation was not reported.	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each concentration was represented by 20 embryos (one embryo per well).	
Domain 5: Outcome As	sessment				
Continued on next page					

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HERO ID: 2298079 Table: 1 of 1

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Study Citation:	Chen, X., X phthalates at	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 Duratio	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:	Vertebrate; H	Fish; Danio rerio; AB Strain; Embryo				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2298079					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	The 24 well plates were described but there 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 embryos.		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and 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 the 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 Quality Determination		Medium				

Study Citation:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and davalanment of zebrafich (Dania raria). Environmental Taxicology and Chemistry 25(0):2304-2404					
Duration: Exposure Route,	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)				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Reproductiv Dibutyl phth 676322	Vertebrate; Fish; <i>Danio rerio</i> ; wild type; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP) 676322				
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	High	The test substance was obtained from Sigma.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%		
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	no significant reported mortality, reproductive output 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 and concentrations were not measured during the study. Exposure was conducted in 7-L glass aquaria.		
	Metric 8:	Consistency of Exposure Administration	High	Vehicle was dimethylformamide at 6 ul/L and static renewal with stock solution was performed daily.		
	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 suitable for the study type, reproductive success was not the primary outcome		
	Metric 11:	Number of Exposure Groups/	Medium	Only two concentrations were used, spacing was adequate		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit		
Domain 4: Test Organis	m					
e	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 most likely the same for control and exposed organisms		
		Conditions		but few details were reported		
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replicates and only 10 organisms were used		
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day.		
		Cont	inued on next pa	ge		

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HERO ID: 676322 Table: 1 of 3

		conti	nued from previo	us page		
Study Citation:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.					
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:	1					
Taxa, Species, Age:	Vertebrate: Fish: <i>Danio rerio</i> : wild type: Adult					
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	676322					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Medium	The details of outcome assessment methodology were not reported		
	Metric 18:	Consistency of Outcome	Medium	outcomes were assessed consistently across study groups but few details were provided		
Assessment						
Domain 6: Confounding	y / Variable Co	ntrol				
Domain of Confounding	Metric 19:	Confounding Variables in Test Design and Procedures	High	All aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day.		
	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. Data Present	Metric 21.	Statistical Methods	High	Statistical analysis was performed and was well described (two way ANOVA followed		
	Metric 21.	Statistical Methous	Ingn	by Duncan's test after testing for normality and homogeneity of variance, or nonpara- metric ANOVA via Kruskal-Wallis followed by Mann-Whitney U test.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for each treatment but not the control group		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	F0 reproduct	tive output, experiment 2				
Overall Quality Determination			Medium			

Study Citation:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.						
Duration: Exposure Route.	Overall Dura Aquatic (fre	ation: > 21 days; Exposure Duration: 11 - 2 shwater): Water: Not determined by study a	21 days uthors (i.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Danio rerio; wild type; Juvenile					
Health Outcome:	Mortality						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	676322						
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	High	The test substance was obtained from Sigma.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%			
Domain 2: Test Design							
2 chian 2. Tost Dosign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	Low	no significant reported mortality, reproductive output not reported			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how F0 organisms were allocated to study groups.			
Domain 3: Exposure Ch	naracterization						
I the second sec	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations and concentrations were not measured during the study. Exposure to the P generation was conducted in 7-L glass aquaria, and the F1 offspring were transferred to 1.5-L glass vessels for observation.			
	Metric 8:	Consistency of Exposure Administration	High	Vehicle was dimethylformamide at 6 ul/L and static renewal with stock solution was performed daily.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	High	F1 embryos were not individually exposed, they were the offspring of P generation exposed for 15 days.			
	Metric 11:	Number of Exposure Groups/	Medium	Only two concentrations were used, spacing was adequate			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were 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 Conditions	Medium	all pretreatment conditions were most likely the same for control and exposed organisms but few details were reported			
	Metric 15:	Number of Organisms and Replicates per Group	Low	No replicates and only 10 F0 organisms were used			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day.			
	Metric 17:	Outcome Assessment Methodology	Medium	The details of outcome assessment methodology were not reported			
	Continued on next page						

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	continued from previous page						
Study Citation:	Ortiz-Zarrag development	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.					
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	thors (i.e., chemi	ical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Danio rerio; wild type; Juvenile					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	676322						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Medium	outcomes were assessed consistently across study groups but few details were 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			
Domain 7: Data Present	tation and Anal	veis					
Domain 7. Data 1 reserv	Metric 21:	Statistical Methods	High	Statistical analysis was performed and was well-described (two-way ANOVA followed by Duncan's test after testing for normality and homogeneity of variance, or nonpara- metric ANOVA via Kruskal-Wallis followed by Mann-Whitney U test.			
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented for only the high concentration			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	F1 survival,	experiment 2					
Overall Quality Determination			Medium				

Study Citation: Duration: Exposure Route, Media Path:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404. 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; F Mechanistic- Dibutyl phth 676322	Vertebrate; Fish; <i>Danio rerio</i> ; wild type; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 576322				
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	High	The test substance was obtained from Sigma.		
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as >98%.		
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	Medium	There was no significant reported mortality. The other measurements appeared 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	The study provided only limited details on the measures taken to appropriately prepare test concentrations and concentrations were not measured during the study. The exposure was conducted in 7-L glass aquaria.		
	Metric 8:	Consistency of Exposure Administration	High	The vehicle solvent was dimethylformamide at 6 ul/L. The static renewal with stock solution was performed daily.		
	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/	Medium	Only two concentrations were used. The exposure spacing was adequate.		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organisi	m					
station of games	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 most likely the same for control and exposed organ- isms but few details were reported		
	Metric 15:	Number of Organisms and	Low	There were no replicates and only 10 organisms were used.		
		Replicates per Group				
Domain 5: Outcome Age	accmont					
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle. They were fed three times per day.		
	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						

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

continued from previous page							
Study Citation:	Ortiz-Zarrag	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and					
Dungton	development	development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (free	snwater); 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; Danio rerio; wild type; Adult					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sig	naling/functi	on-Oxidative stress (including redox biology)			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	676322						
Domain		Metric	Rating	Comments			
		_					
Domain 6: Confounding	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test	High	All aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10			
		Design and Procedures		light cycle. They were fed three times per day.			
	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					
Domain 7. Dua Presen	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 unevpected outcomes			
		Explanation of Onexpected Outcomes	man				
Additional Comments:	This evaluati	ion is for experiment 2.					
Overall Quality Determination		High					

Study Citation:	Chen, P., Li,	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with						
Duration:	Zebrafish (Da Overall Dura	verall 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:		Vertebrate; Fish; Danio rerio; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age:	Vertebrate; F							
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sig	gnaling/functi	on				
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	2810885							
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	The test substance identity was verified by GC-MS.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >98%.				
Demain 2. Test Design								
Domain 2: Test Design	Matria 4	Nagative Controls	High	Study outhous reported using an appropriate consumpt possible control group (column				
	Meure 4.	Negative Controls	Ingn	control containing 0.005% DMSO).				
	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 2. Eurocum Ch	anastanization							
Domain 5: Exposure Ch	Metric 7	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in				
	Wietrie 7.	Preparation	Ingn	adequate detail.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups using a semi-static				
		Administration	8	method with half-renewal daily.				
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies and				
	Matric 10:	Concentration Exposure Duration and Erequency	High	The duration of avacuum was suitable for the study type				
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments were used, but they were at a suitable range				
	Wieure 11.	Spacing of Exposure Levels	Medium	Only two treatments were used, but they were at a suitable range.				
	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 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	There were 7-12 fish per tank with four replicates.				
		Replicates per Group						
Domain 5: Outcome As	sessment							

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Environmental Hazard Evaluation

HERO ID: 2816885 Table: 1 of 6

continued from previous page							
Study Citation:	Chen, P., Li, zebrafish (Da	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526.					
Duration:	Overall Dura	tion: > 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; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cell sign	naling/function	on			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels were not reported.			
	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	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	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quality Determination			High				

Study Citation: Duration: Exposure Route, Media, Path:	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526. 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; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development	Development/Growth						
Chemical:	Dibutyl phtha	Dibutyl phthalate (DBP)						
HERO ID:	2816885							
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	High	The test substance identity was verified by GC-MS.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (solvent control containing 0.005% DMSO).				
	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 3. Exposure en	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 using a semi-static method with half-renewal daily.				
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type				
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments but at a suitable range				
	Matria 12.	Spacing of Exposure Levels	Uiah	Even source concentrations were halow the water solubility limit				
	Mettic 12.	Testing at of Below Solubility Limit	підії	Exposure concentrations were below the water solubility infint				
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	Low	The study did not report whether test organisms were acclimatized				
	Metric 15:	Conditions Number of Organisms and	Medium	7-12 fish per tank with 4 replicates				
		Replicates per Group						
Domain 5: Outcome Ass	sessment							
2 sinum 5. Sutcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest				
		Cont	inued on nex	t page				

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HERO ID: 2816885 Table: 2 of 6

continued from previous page							
Study Citation:	Chen, P., Li,	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α-ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with					
	zebrafish (Da	zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526.					
Duration:	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; F	Fish; Danio rerio; TU strain; Not Applicabl	e (e.g., fungi	or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Domain 6: Confounding	y / Variable Cou	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			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups			
Damain 7. Data Durant							
Domain 7: Data Present	Matria 21.	ysis Statistical Mathada	High				
	Metric 21:	Statistical Methods	High Li -h	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	I nere were no unexpected outcomes			
Additional Comments:	length weig	ht condition index					
raditional Comments.	iongen, worg						

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path:	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526. 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>Danio rerio</i> : TU strain: Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality							
Chemical:	Dibutyl phtha	alate (DBP)						
HERO ID:	2816885							
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	High	The test substance identity was verified by GC-MS.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%				
Domain 2: Test Design								
2 oniani 21 1000 2 001gn	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (solvent control containing 0.005% DMSO).				
	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	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 using a semi-static method with half-renewal daily.				
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type				
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments but at a suitable range				
	Matria 12	Spacing of Exposure Levels	Uiah	Even over a concentrations were below the water calubility limit				
		resultg at or below Solubility Limit	nigii	Exposure concentrations were below the water solubility fimit				
Domain 4: Test Organis	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	Low	The study did not report whether test organisms were acclimatized				
	Metric 15:	Conditions Number of Organisms and	Medium	7-12 fish per tank with 4 replicates				
		Replicates per Group						
Domain 5: Outcome Ass	essment							
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported				
	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: 2816885 Table: 3 of 6

	continued from previous page					
Study Citation:	Chen, P., Li,	S., Liu, L., Xu, N. (2015). Long-term effe	cts of binary	mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with		
	zebrafish (D	anio rerio). Environmental Toxicology and	Chemistry 3	34(3):518-526.		
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; F	Fish; Danio rerio; TU strain; Not Applicabl	e (e.g., fungi	i or algae studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2816885					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	mortalities were checked daily		
		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		
	M (20	Design and Procedures	M P			
	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 (see supplemental data sheet)		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	None					
Overall Quality Determination			High			

Study Citation: Duration: Exposure Route, Media. Path:	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526. 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; Fish; <i>Danio rerio</i> ; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported Hepatic/Liver Dibutyl phthalate (DBP)						
HERO ID:	2816885						
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	High	The test substance identity was verified by GC-MS.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (solvent control containing 0.005% DMSO).			
	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 Ch	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 using a semi-static method with half-renewal daily.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments but at a suitable range			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organis	m						
Zomuni i. 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			
	Metric 15:	Conditions Number of Organisms and	Medium	7-12 fish per tank with 4 replicates			
		Replicates per Group					
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
		Cont	tinued on nex	t page			

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HERO ID: 2816885 Table: 4 of 6

continued from previous page					
Study Citation:	Chen, P., Li,	S., Liu, L., Xu, N. (2015). Long-term effe	cts of binary	mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with	
Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526. 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> ; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported Hepatic/Liver				
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	2816885				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups	
		Assessment			
Domain 6: Confounding	y / Variable Cou	atrol			
Domain 0. Comounding	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	
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 form is	to account for the liver histology.			
Overall Quality Determination		High			

Study Citation:	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17 α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with						
Duration	zebrafish (Da	zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526.					
Duration: Evensue Douto	A quetio (free	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)					
Exposure Noure, 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)						
Tava Spacias Aga	Vartabrata: Fish: Davia ravia: TU strain: Nat Applicable (a.g., fungi or algae studiae) or Nat Bapartad						
Health Outcome	Reproductive	Demoduative/Terretegenic					
Chemical.	Dibutyl phth	Dibutyl nothalate (DBP)					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	High	The test substance identity was verified by GC-MS.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (solvent control containing 0.005% DMSO).			
	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 Cha	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 using a semi-static method with half-renewal daily.			
	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 was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments but at a suitable range			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organisr	n Maria 12		TT' 1				
	Metric 13:	Test Organism Characteristics	Hign	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Conditions	LOW	The study did not report whether test organisms were acclimatized			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	7-12 fish per tank with 4 replicates			
		Repleates per Gloup					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	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: 2816885 Table: 5 of 6

		conti	ued from p	revious page			
Study Citation:	Chen, P., Li, zebrafish (Da	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with ebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526.					
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	ish; Danio rerio; TU strain; Not Applicabl	e (e.g., fungi	or algae studies) or Not Reported			
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
	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	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	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:	Additional Comments: This form is to account for the gonad histology and the sex ratio determination, both of which are reproductive outcomes.						
		•					

Overall Quality Determination

High

Study Citation:	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with zebrafish (Danio rerio). Environmental Toxicology and Chemistry 34(3):518-526.						
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: Health Outcome: Chemical:	Vertebrate; F Respiratory Dibutyl phth	Vertebrate; Fish; <i>Danio rerio</i> ; TU strain; Not Applicable (e.g., fungi or algae studies) or Not Reported Respiratory					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	High	The test substance identity was verified by GC-MS.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (solvent control containing 0.005% DMSO).			
	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 Cha	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 using a semi-static method with half-renewal daily.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Only two treatments but at a suitable range			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
			U	· · · ·			
Domain 4: Test Organism	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	Low	The study did not report whether test organisms were acclimatized			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	7-12 fish per tank with 4 replicates			
		Replicates per Gloup					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	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: 2816885 Table: 6 of 6

continued from previous page							
Study Citation:	Chen, P., Li,	Chen, P., Li, S., Liu, L., Xu, N. (2015). Long-term effects of binary mixtures of 17α -ethinyl estradiol and dibutyl phthalate in a partial life-cycle test with ebrafish (Danio regio). Environmental Toxicology and Chemistry 34(3):518-526					
Duration:	Overall Dura	Δr					
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:	i iquitio (iio)						
Taxa, Species, Age:	Vertebrate; F	ish; <i>Danio rerio</i> ; TU strain; Not Applicabl	e (e.g., fungi	or algae studies) or Not Reported			
Health Outcome:	Respiratory						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816885						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Domain 6: Confounding	y / Variable Cou	atrol					
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			
	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	Metric 21.	Statistical Methods	High	Statistical methods were adequately 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			
	metric 23.	Explanation of Onexpected Outcomes	mgn				
Additional Comments:	This form is	to account for the gill histology.					
Overall Qualit	ty Detern	nination	High				

Study Citation:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and						
Duration: Exposure Route, Media, Path:	development 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; Danio rerio; wild type; Embryo					
Health Outcome:	Developmen	tt/Growth					
Chemical: HFRO ID:	Dibutyl phth	ialate (DBP)					
Domain	070322	Metric	Rating	Comments			
Domain 1: Test Substan	ce		Tutting				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only			
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >98%			
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	Low	mortality rate of 20-30% 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, and concentrations were not measured during the study. Exposure was conducted in 2-L glass vessels			
	Metric 8:	Consistency of Exposure	High	Vehicle was dimethylformamide at 6 ul/L and static renewal with stock solution was performed daily			
	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 suitable for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Only two concentrations were used, spacing was adequate			
	Metric 12:	Spacing of Exposure Levels 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 adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	all pretreatment conditions were most likely the same for control and exposed organisms but few details were reported			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Two replicates and enough organisms to allow subsampling			
Domain 5: Outcome As	sessment						
· · · · · · · · · · · · · · · · · · ·	Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day. if adequate			
		Conti	nued on next pa	ge			

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HERO ID: 676322 Table: 1 of 3

		contir	ued from previ	ous page		
Study Citation: Duration:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404. Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route, Madia 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: Fish: Danio rerio: wild type: Embryo					
Health Outcome:	Development/Growth					
Chemical: HERO ID:	Dibutyl phth 676322	nalate (DBP)				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology were adequate		
	Metric 18:	Consistency of Outcome Assessment	Low	It was unclear if outcomes were assessed consistently across study groups, few details were provided		
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		
	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 analysis was performed and was well-described (two-way ANOVA followed by Duncan's test after testing for normality and homogeneity of variance, or nonpara- metric ANOVA via Kruskal-Wallis followed by Mann-Whitney U test.		
	Metric 22:	Reporting of Data	Low	Data was not presented for each treatment		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	experiment	1				

Study Citation:	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.						
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 Mechanistic- Dibutyl phth 676322	Vertebrate; Fish; <i>Danio rerio</i> ; wild type; Embryo Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 676322					
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	High	The test substance was obtained from Sigma.			
	Metric 3:	Test Substance Purity	High	Chemical purity 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	Medium	Reported mortality of 20-30% in both controls and DBP-exposed treatments.			
	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 concentrations were not measured during the study. Exposure was conducted in 2-L glass vessels.			
	Metric 8:	Consistency of Exposure Administration	High	Vehicle was dimethylformamide at 6 ul/L and static renewal with stock solution was performed daily.			
	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/	Medium	Only two concentrations were used, spacing was adequate			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
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	Medium	all pretreatment conditions were most likely the same for control and exposed organisms but few details were reported			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	two replicates and enough organisms to make subsampling possible			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day.			
	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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		conti	nued from p	revious page			
Study Citation:	Ortiz-Zarrag developmen	Drtiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and levelopment of zebrafish (Danio rerio). Environmental Toxicology and Chemistry 25(9):2394-2404.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	l 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:							
Taxa, Species, Age:	Vertebrate; I	Fish; Danio rerio; wild type; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sig	naling/functi	on-Oxidative stress (including redox biology)			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	676322						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test Design and Procedures	High	All aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle and fed three times per day.			
	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 and at several time points			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	experiment	1					
Overall Qualit	ty Deterr	nination	High				

Study Citation:	Ortiz-Zarrag	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and					
Duration:	development Overall Dura	verall 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	Vertahrate: Fish: Dania raria: wild tupe: Embryo					
Health Outcome:	Mortality	isit, Dano rerio, wild type, Emoryo					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	676322						
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	The test substance was obtained from Sigma			
	Metric 3:	Test Substance Purity	High	The chemical purity 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	A mortality rate of 20-30% was reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	Aracterization	Europimantal System/Test Madia	Low				
	Metric 7.	Preparation	Low	test concentrations, and concentrations were not measured during the study. The expo- sure was conducted in 2-L glass vessels.			
	Metric 8:	Consistency of Exposure Administration	High	The vehicle solvent was dimethylformamide at 6 ul/L. Static renewal with stock solution was performed daily.			
	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 suitable for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Only two concentrations were used. The exposure spacing was adequate.			
		Spacing of Exposure Levels					
	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 adequately described and were obtained from a reliable source.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-			
	Metric 15	Conditions Number of Organisms and	Medium	isms, but few details were reported. Two replicates were used along with enough organisms to allow for subsampling			
	Wieute 15.	Replicates per Group	Wiedium	Two replicates were used, along with chough organishis to allow for subsampling.			
Domain 5: Outcome Ac	recoment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Aquaria were maintained at 28C with conditions as appropriate for D. rerio at a 14:10 light cycle. They were fed three times per day.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was adequate.			
	Continued on next page						

continued from previous page							
Study Citation:	Ortiz-Zarrag	Ortiz-Zarragoitia, M., Trant, J. M., Cajaravillet, M. P. (2006). Effects of dibutylphthalate and ethynylestradiol on liver peroxisomes, reproduction, and development of zebrafish (Danio ratio). Environmental Toxicology and Chamistry 25(0):394-2404					
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 untake route)					
Media, Path:	riquitie (ire.	sirvater), water, not determined by study ad	unors (i.e., enem	ear of interest in exposure water, but anable to determine exact aptake route)			
Taxa, Species, Age:	Vertebrate: F	Fish: Danio rerio: wild type: Embryo					
Health Outcome	Mortality	ish, Dunio rerio, wild type, Emoryo					
Chemical.	Dibutyl phth	alate (DBP)					
HERO ID:	676322						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Medium	Outcomes were assessed consistently across study groups, but few details were pro- vided.			
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			
	Metric 20.	Design and Procedures Outcomes Unrelated to Exposure	Medium	Conditions.			
	Methe 20.	Sucomes chickaed to Exposure	Wiedrum	There was no information in the study to suggest unreferences among groups.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical analysis was performed and was well-described (two-way ANOVA followed by Duncan's test after testing for normality and homogeneity of variance, or nonpara- metric ANOVA via Kruskal-Wallis followed by Mann-Whitney U test).			
	Metric 22:	Reporting of Data	Low	Data was not presented for each treatment.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluat	ion is for experiment 1.					
Overall Quali	ty Detern	nination	Medium				

Study Citation: Duration:	Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environ- mental Toxicology and Chemistry 30(6):1338-1345. Overall Duration: > 21 days; Exposure Duration: > 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; Fish; Gasterosteus aculeataus; males; Adult							
Health Outcome:	Behavioral	Behavioral						
Chemical:	Dibutyl phth	alate (DBP)						
Demain	/88294	M-4-:-	Datina	Commente				
Domain Domain 1: Test Substan		Metric	Rating	Comments				
Domain 1. Test Substan	Metric 1.	Test Substance Identity	Low	The test substance was identified by name only				
	Metric 2:	Test Substance Source	High	DBP was sourced from Fisher Scientific and was analytically verified via GC/MS				
	Metric 3:	Test Substance Purity	Low	The nurity of the DBP was not reported				
	metric 5.		Low					
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	The study authors reported using a negative control with the solvent used in testing.				
	Metric 5:	Negative Control Response	High	The solvent control response was reported in Table 4 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Medium	Study authors reported the fish were allocated randomly into study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Medium	Little detail was provided on how the stock solutions were prepared. The system was flow through. Concentrations of test substance were measured during the study every 3-4 days. Exposure was conducted in 3.8L tanks (presumably made of glass, as is standard for aquaria).				
	Metric 8:	Consistency of Exposure Administration	Medium	There were eight fish per treatment group. A flow through system was used for all tanks. Water flow was the same for all tanks. The concentrations of DBP detected were not close to the nominal concentrations, creating some concern about consistency.				
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured every 3-4 days. The reported concentrations were not close to the nominal concentrations, but study authors reported the endpoints were based on the measured concentrations.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be for 22 days. This was adequate time to ob- serve nesting behavior in all the exposure concentrations.				
	Metric 11:	Number of Exposure Groups/	Medium	There were only two exposure groups plus a control. This was adequate to observe a response, but more exposure levels may have yielded more data. Spacing was adequate				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using methanol as a vehicle solvent.				
			8					
Domain 4: Test Organis	m							
-	Metric 13:	Test Organism Characteristics	Low	The source of the fish was reported to be ponds near Hook, Hampshire, United King- dom. This creates significant concern about the health of the organisms prior to testing.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions prior to the start of the study. The reported 10-day "acclimation period" was conducted during the test, while exposure to DBP was occurring. Therefore it cannot be considered a true acclimation.				
		Conti	nued on next pa	ge				

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Dibutyl Phthalate

HERO ID: 788294 Table: 1 of 3

		continu	ued from previ	ious page		
Study Citation: Duration: Exposure Route,	Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environ- mental Toxicology and Chemistry 30(6):1338-1345. 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; Fish; Gasterosteus aculeataus; males; Adult					
Health Outcome:	Behavioral					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	788294					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Eight fish were exposed per treatment (including control) for a total of 24 fish, exposed in individual containers.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Fish were housed in 3.8L tanks in dechlorinated water and fed three times a day. Prior to treatment they were held at 8L:16hD photoperiod at 18-20C, which was winter conditions to lead to sexual maturity. They were switched to 16L:8D with higher light intensity to bring the fish to full sexual maturity.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–nesting behavior.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Males were observed for nesting behavior by photographs of nest building progress every two days. Threads and gravel for nesting material were added to all exposure groups on day 10.		
Domain 6: Confoundin	a / Variable Cor	atrol				
	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. It was not reported if the organisms were acclimated to test conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	Study authors reported a fish for one treatment exhibited no nesting behavior and was removed from the data because it was considered an outlier. They also discussed any potential contamination by other phthalates in the test media.		
Domain 7: Data Presen	tation and Anal	veje				
	Metric 21:	Statistical Methods	High	Statistical analyses were described in the "Statistical Analysis" section and were de- scribed in detail.		
	Metric 22:	Reporting of Data	High	Data for the exposure related finding and the control response were reported in Figure 4.		
	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 nesting behavio	or of G. aculeat	as during exposure to DBP. The behavior outcome was chosen for this portion.		
Overall Ouali	tv Detern	nination	Medium			
Overall Quali	ty Detern	nination	Medium	l		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environ- mental Toxicology and Chemistry 30(6):1338-1345. 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>Gasterosteus aculeataus</i> ; males; Adult Development/Growth Dibutyl phthalate (DBP) 788294				
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	High	DBP was sourced from Fisher Scientific and was analytically verified via GC/MS.	
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	The study authors reported using a negative control with the solvent used in testing.	
	Metric 5:	Negative Control Response	Low	The length, weight, and GSI of the control group fish were not specifically reported.	
	Metric 6:	Randomized Allocation	Medium	Study authors reported the fish were allocated randomly into study groups.	
Domain 3: Exposure Ch	aracterization				
·	Metric 7:	Experimental System/Test Media Preparation	Medium	Little detail was provided on how the stock solutions were prepared. The system was flow through. Concentrations of test substance were measured during the study every 3-4 days. Exposure was conducted in 3.8L tanks (presumably made of glass, as is standard for aquaria).	
	Metric 8:	Consistency of Exposure Administration	Medium	There were 8 fish per treatment group. A flow through system was used for all tanks. Water flow was the same for all tanks. The concentrations of DBP detected were not close to the nominal concentrations, creating some concern about consistency.	
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured every 3-4 days. The reported concentrations were not close to the nominal concentrations, but study authors reported the endpoints were based on the measured concentrations.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be for 22 days. This was adequate time to ob- serve changes in growth and development.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only 2 exposure groups plus a control. This was adequate to observe a re- sponse, but more exposure levels may have yielded more data. Spacing was adequate.	
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using methanol as a vehicle solvent.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Low	The source of the fish was reported to be ponds near Hook, Hampshire, United King- dom. This creates significant concern about the health of the organisms prior to testing.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions prior to the start of the study. The reported 10-day "acclimation period" was conducted during the test, while exposure to DBP was occurring. Therefore it cannot be considered a true acclimation.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	8 fish were exposed per treatment (including control) for a total of 24 fish, exposed in individual containers.	

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Dibutyl Phthalate

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Study Citation:	Aoki, K. A., mental Toxic	Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environ- mental Toxicology and Chemistry 30(6):1338-1345.						
Duration:	Overall Dura	Verall 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	Fish; Gasterosteus aculeataus; males; Adult						
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	788294							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	ssessment							
	Metric 16:	Adequacy of Test Conditions	High	Fish were housed in 3.8L tanks in dechlorinated water and fed 3 times daily. Prior to treatment they were held at 8L:16hD photoperiod at 18-20C, which was winter conditions to lead to sexual maturity. They were switched to 16L:8D with higher light intensity to bring the fish to full sexual maturity.				
	Metric 17:	Outcome Assessment Methodology	Low	Methodology to assess length, weight and GSI was not described.				
	Metric 18:	Consistency of Outcome	Low	Since methods were not described for assessing length, weight, and GSI it is uncertain				
		Assessment		whether consistency occurred across all study groups.				
Domain 6: Confoundin	α / 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. It was not reported if the organisms were acclimated to test conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest any differences in organisms among groups that would impact the outcome assessment.				
Domain 7: Data Presen	tation and Anal	vsis						
	Metric 21:	Statistical Methods	High	Statistical analyses were described in the "Statistical Analysis" section and were de- scribed in detail.				
	Metric 22:	Reporting of Data	Medium	Length, weight, and GSI were briefly described in text in the results section, but there was no data shown in tables or figures. There were no significant differences found with these parameters.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				
Additional Comments:	This evaluat	ion is for Development/Growth assessing th	e analysis of leng	gth, weight, and GSI.				

Overall Quality Determination

Medium
Study Citation: Duration: Exposure Route, Media, Path:	Aoki, K. A., Harris, C. A., Katsiadaki, I., Sumpter, J. P. (2011). Evidence suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environmental Toxicology and Chemistry 30(6):1338-1345. 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 Mechanistic- Dibutyl phth 788294	ish; <i>Gasterosteus aculeataus</i> ; males; Adult Biomarkers (exposure and effect)-Endocrir alate (DBP)	ne toxicity-Repro	oductive/Teratogenic	
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	High	DBP was sourced from Fisher Scientific and was analytically verified via GC/MS.	
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.	
Domain 2: Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	The study authors reported using a negative control with the solvent used in testing.	
	Metric 5:	Negative Control Response	High	The solvent control response was reported in Figures 2 and 3 and was adequate for the outcome of interest.	
	Metric 6:	Randomized Allocation	Medium	Study authors reported the fish were allocated randomly into study groups.	
Domain 3: Exposure Ch	aracterization Metric 7: Metric 8: Metric 9:	Experimental System/Test Media Preparation Consistency of Exposure Administration Measurement of Test Substance	Medium Medium High	Little detail was provided on how the stock solutions were prepared. The system was flow through. Concentrations of test substance were measured during the study every 3- 4 days. Exposure was conducted in 3.8L tanks (presumably made of glass, as is standard for aquaria). There were 8 fish per treatment group. A flow through system was used for all tanks. Water flow was the same for all tanks. The concentrations of DBP detected were not close to the nominal concentrations, creating some concern about consistency. Test concentrations were measured every 3-4 days. The reported concentrations were not close to the nominal concentrations but study authors reported the endpoints were	
	Metric 10:	Exposure Duration and Frequency	High	based on the measured concentrations. The exposure duration was reported to be for 22 days. This was adequate time to ob-	
	Metric 11:	Number of Exposure Groups/	Medium	serve nesting behavior in all the exposure concentration. There were only 2 exposure groups plus a control. This was adequate to observe a re- sponse, but more exposure levels may have yielded more data. Spacing was adequate	
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using methanol as a vehicle solvent.	
Domain 4: Test Organis	m Metric 13:	Test Organism Characteristics	Low	The source of the fish was reported to be ponds near Hook, Hampshire, United King- dom. This creates significant concern about the health of the organisms prior to testing.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions prior to the start of the study. The reported 10-day "acclimation period" was conducted during the test, while exposure to DBP was occurring. Therefore it cannot be considered a true acclimation.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	8 fish were exposed per treatment (including control) for a total of 24 fish, exposed in individual containers.	
Continued on next page					

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HERO ID: 788294 Table: 3 of 3

		contin	ued from previ	ous page	
Study Citation:	Aoki, K. A., mental Toxic	Harris, C. A., Katsiadaki, I., Sumpter, J. P. cology and Chemistry 30(6):1338-1345.	(2011). Evidend	e suggesting that di-n-butyl phthalate has antiandrogenic effects in fish. Environ-	
Duration:	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:					
l'axa, Species, Age:	Vertebrate; Fish; <i>Gasterosteus aculeataus</i> ; males; Adult				
Health Outcome:	Mechanistic-	-Biomarkers (exposure and effect)-Endocrine	e toxicity-Repro	ductive/Teratogenic	
Unemical:	Dibutyl phth	alate (DBP)			
	100294		D. d		
Domain		Metric	Rating	Comments	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Fish were housed in 3.8L tanks in dechlorinated water and fed 3 times daily. Prior to treatment they were held at 8L:16hD photoperiod at 18-20C, which was winter conditions to lead to sexual maturity. They were switched to 16L:8D with higher light intensity to bring the fish to full sexual maturity.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–changes in plasma concentrations of 11 ketotestosterone and testosterone, as well as spiggin production. StAR and 3B-HSD gene expression assessment was also addressed.	
	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			
Johan o. 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. It was not reported if the organisms were acclimated to test conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	High	Study authors reported a fish for one treatment exhibited no nesting behavior and was removed from the data because it was considered an outlier. They also discussed any potential contamination by other phthalates in the test media.	
Domain 7: Data Presenta	ation and Anal	vsis			
	Metric 21:	Statistical Methods	High	Statistical analyses were described in the "Statistical Analysis" section and were de- scribed in detail.	
	Metric 22:	Reporting of Data	Medium	Data for the exposure related finding and the control response were reported in Figures 2 and 3. Gene expression data was reported in the text only.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	25th and 75th percentiles were reported surrounding the mean in all figures.	
Additional Comments:	This portion of the evaluation was on the effect of DBP on spiggen prodcution, ketotestosterone and testosterone plasma levels, and steroidogenic gene expression. Mechanistic outcomes were selected under biomarkers, reproduction, and endocrine system.				

Study Citation: Duration: Exposure Route,	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)					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Dibutyl phth 1321996	Fish; <i>Lepomis macrochirus</i> ; Juvenile alate (DBP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The test substance was reported without a CASRN.			
	Metric 2:	Test Substance Source	Low	The source was reported as provided by a 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	The test substance was at least 95% pure.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A negative control was reported.			
	Metric 5:	Negative Control Response	High	The negative control response was acceptable.			
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to pre- vent volatilization were not reported.			
	Metric 8:	Consistency of Exposure	High	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 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 exposure were appropriate for this 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 for this 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 As	sessment						

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Environmental Hazard Evaluation

HERO ID: 1321996 Table: 1 of 1

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):1569-15	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:	Vertebrate; Fish; Lepomis macrochirus; Juvenile						
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
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 Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.			
		Design and Procedures	C				
	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			
		Explanation of onexpected outcomes	111511				
Additional Comments:	None						
Overall Quality Determination		High					

Study Citation: Duration: Exposure Pouto	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) Aquetic (freehwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)							
Media. Path:	Aquatic (free	Aquatic (reshwater), 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; Lepomis macrochirus; Not Applicable	e (e.g., fungi o	or algae studies) or Not Reported				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	1316201							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	Metric 1:	Test Substance Identity	High	The test substance- Dibutyl phthalate (DBP) was identified by chemical name and CASRN (84-74-2).				
	Metric 2:	Test Substance Source	Low	The source was not reported and the test substance identity was not analytically verified by the performing labortory.				
	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 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	Low	The study provided only limited details on the measures taken to appropriately prepare				
		Preparation		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 study authors and adequate to address the purpose of the study. For Dibutyl phthalate (DBP), a preliminary test was conducted, which indicated that it was not toxic below the water solubility limit. A corroborative test was then conducted exposing the bluegill to a single replicated concentration of Dibutyl phthalate (DBP) 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 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 Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms.				
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		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media Path:	Bionomics,, Overall Dura Aquatic (free	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 [.] F	Vertebrate: Fish: Lepomis macrochirus: Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome	Mortality	isii, Leponus nucroentrus, ivot Applicable	(e.g., rungi	or argae studies) of Not Reported			
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1316201	1316201					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten bluegill in each test jar, and they were tested in duplicates.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to maintenance of health. The 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 & Confounding	Variable Co	ntanl					
Domani 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	voic					
Domain 7. Data Present	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 were reported based on a corroborative test (following negative findings from a preliminary test at concentrations below the limit of water solubility) conducted by exposing bluegills to a single replicated concentration of Dibutyl phthalate (DBP) representing it's limit of water solubility.						
Overall Qualit	ty Detern	nination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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. 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 Dibutyl phthalate (DBP) 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					
	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 groups was not 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	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/	Low	No information is provided on the number of exposure groups and spacing of exposure	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	levels, but cited methods suggest using a minimum of five treatment levels. The authors reported this as "precipitate." However, the highest reported LC50 value 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 Organis	m				
Domain 4. Test Organis	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.	
		Conti	inued on next pa	ge	

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Dibutyl Phthalate

HERO ID: 18064 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation:	Buccafusco, Contaminati	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					
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:	1						
Taxa, Species, Age:	Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	18064						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Ten fish were used but no replicates were reported.			
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	Low	Details regarding the execution of the study protocol for outcome assessment were not 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	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.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described (method of moving average angles or Wilcoxon log probit).			
	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 tables.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Occurrence of unexpected outcomes was not addressed.			
Additional Comments:	None						

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media Path:	Aktiengesellschaft,, BASF (1989). Report on the study of the acute toxicity of dibutylphthalat on the Golden Orfe (Leuciscus idus L., golden variety). 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:	Vertebrate; H Mortality Dibutyl phth	Fish; <i>Leuciscus idus L</i> .; Golden variety; Not	Applicable (e.g.	, fungi or algae studies) or Not Reported			
HERO ID:	10817969	10817969					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported, though BASF Aktiengeselschaft is the report- ing laboratory.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be greater than 99.0%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a solvent control that contained 1mL/L of acetone. A dilution water only control was not reported.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in the table on page 7 of the report and was appropriate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported if the fish were randomly allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 9. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The test substance was prepared by dissolving it in acetone at a rate of 1.0g/mL, and this was added to the test water. Detailed methods were not provided. The system was reported to be a flow-through system, but flow rate was not reported. The test vessels were 10-L glass aquaria.			
	Metric 8:	Consistency of Exposure Administration	Low	Test aquaria were reported to be 30 x 22 x 24cm, and fish were loaded at a rate of 3.4g of fish per liter of water. Flow rates and preparation of the test concentrations were not described in detail. All exposures were conducted at 19-20C with a 16L:8D photoperiod.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors did not report if the test concentrations were measured 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 a fish acute toxicity test.			
	Metric 11:	Number of Exposure Groups/	Low	There were six exposure levels. However, only the highest concentration had mortalities.			
		Spacing of Exposure Levels		the 4.64mg/L and the 10mg/L exposure levels.			
	Metric 12:	Testing at or Below Solubility Limit	High	All of the test concentrations were below the water solubility limit for DBP. A vehicle solvent was also used, and the solvent control results were appropriate.			
Domain 4. Test Organic	m						
	Metric 13:	Test Organism Characteristics	Medium	The fish were reported to be from Fischzucht Paul Eggers D-2354 Hohenwestedt, FRG. The age was not reported, but they were reported to be 7.3cm long and weighed 3.4g.			
		Contin	nued on next pa	ge			

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		contin	ued from previ	ous page		
Study Citation: Duration: Exposure Route, Media. Path:	Aktiengesellschaft, BASF (1989). Report on the study of the acute toxicity of dibutylphthalat on the Golden Orfe (Leuciscus idus L., golden variety). 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>Leuciscus idus L</i> .; Golden variety; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 10817969					
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment	High	The fish were held for four months prior to testing and were acclimated to test condi- tions for three days		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test concentration. The number of replicates was not reported.		
Demain 5: Outerme Are						
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	The fish were kept at a loading rate of 3.4g of fish per liter of water. They were fed ad libitum until one day before the start of the test. They had a 16L:8D photoperiod. The water used was reconstituted fresh water, and the water quality characteristics were reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality. LC50 values for 1, 4, 24, 48, 72, and 96h were reported.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortalities were assessed at 1h, 4h, 24h, 48h, 72h, and 96h.		
Domain 6: Confounding	/ 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. The organisms were accli- mated 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 the exposure.		
Domain 7: Data Presenta	ation and Analy	vsis				
Domain 7. Data Presenta	Metric 21:	Statistical Methods	High	Study authors reported using probit analysis to estimate LC50 values.		
	Metric 22:	Reporting of Data	High	Data was reported for all exposure levels and the controls on page 7 and was adequate for the outcomes of interest. LC50 values for each observation interval were reported on pages 11-16.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This evaluati outcome of i	on was on the acute toxicity of DBP on gold nterest.	len orfe. Study a	uthors calculated the LC50 values for 1, 4, 24, 48, 72, and 96h. Mortality was the		
Overall Qualit	y Detern	nination	Medium			

Study Citation:	Aktiengesellschaft, BASF (1989). Report on the study of the acute toxicity of dibutylphthalat on the Golden Orfe (Leuciscus idus L., golden variety).							
Duration: 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:	riquite (ire	invaler), water, not determined by study and	ions (n.e., enemieur or					
Taxa, Species, Age:	Vertebrate; Fish; Leuciscus idus L.; Golden variety; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Behavioral							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	10817969							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce		Ţ					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported, though BASF Aktiengeselschaft is the report- ing laboratory.				
	Metric 3:	Test Substance Purity	High	The purity was reported to be greater than 99.0%.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a solvent control only that contained 1mL/L of ace- tone. A dilution water only control was not reported.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in the table on page 7, where symptoms were reported, of the report and was appropriate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported if the fish were randomly allocated into study groups.				
Domain 3: Exposure Ch	aracterization		т					
	Metric /:	Preparation	Low	The test substance was prepared by dissolving it in acetone at a rate of 1.0g/mL, and this was added to the test water. Detailed methods were not provided. The system was reported to be flow-through system, but flow rate was not reported. 10-L glass aguaria.				
	Metric 8:	Consistency of Exposure Administration	Low	Test aquaria were reported to be 30 x 22 x 24cm, and fish were loaded at a rate of 3.4g of fish per liter of water. Flow rates and preparation of the test concentrations were not described in detail. All exposures were conducted at 19-20C with a 16L:8D photoperiod.				
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors did not report if the test concentrations were measured 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 a fish acute toxicity test.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were 6 exposure levels. Only the two highest concentrations were reported to have changes in behavior observed. This indicates that a different spacing may have yielded more data, especially between the 4.64mg/L and the 10mg/L exposure levels.				
	Metric 12:	Testing at or Below Solubility Limit	High	All of the test concentrations were below the water solubility limit of the DBP. A vehicle solvent was also used, and the solvent control results were appropriate.				
Domain 4: Test Organis	m							
C	Metric 13:	Test Organism Characteristics	Medium	The fish were reported to be from Fischzucht Paul Eggers D-2354 Hohenwestedt, FRG. The age was not reported, but they were reported to be 7.3cm long and weighed 3.4g.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were held for 4 months prior to testing and were acclimated to test conditions for 3 days.				
		Со	ntinued on next page	·				

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10817969 Table: 2 of 2

		col	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path:	Aktiengesell Overall Dura Aquatic (free	Aktiengesellschaft,, BASF (1989). Report on the study of the acute toxicity of dibutylphthalat on the Golden Orfe (Leuciscus idus L., golden variety). 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>Leuciscus idus L</i> .; Golden variety; Not Applicable (e.g., fungi or algae studies) or Not Reported Behavioral Dibutyl phthalate (DBP) 10817969					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test concentration. The number of replicates was not reported.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	The fish were kept at a loading rate of 3.4g of fish per liter of water. They were fed ad libitum until one day before the start of the test. They had a 16L:8D photoperiod. The water used was reconstituted fresh water, and the water quality characteristics were reported.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was not reported how the fish were assessed for behavioral changes.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol were not reported for the behav- ioral portion of the study.		
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 or other factors that could influence the outcome assessment. The organisms were accli- mated 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.		
Demain 7. Data Dreamt	- 4 ¹					
Domain 7: Data Present	Metric 21:	Statistical Methods	Uninformative	Study authors did not report conducting statistical analysis on any of the behavioral changes that were noted. Behavioral outcomes were not quantified in any manner; they were simply noted in the table on page 7 of the report.		
	Metric 22:	Reporting of Data	Medium	Data was reported for every exposure level in the table on page 7. Note that the behav- ioral outcomes were not quantified, they were simply noted in this table.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	Additional Comments: This evaluation was on the acute toxicity of DBP on golden orfe. Study authors reported on behavioral changes in the fish in the table on page 7 of the report. These behavioral changes were simply noted, not quantified. This meant statistical analysis was not conducted for this outcome, thus the unacceptable rating.					
Overall Quality Determination Uninformative						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bhatia, H., F (Melanotaen Overall Dura Aquatic (fres Vertebrate; F Hepatic/Live Dibutyl phth 1639196	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344. 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) Vertebrate; Fish; <i>Melanotaenia fluviatilis</i> ; Adult Hepatic/Liver Dibutyl phthalate (DBP) 1639196					
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	High	The test substance identity was verified by GC-MS.			
	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, and a			
	Metric 5:	Negative Control Response	Medium	No mortality in any treatments reported, some growth results were only in text as not significant.			
	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. Exposure was conducted in glass aquaria.			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported and exposures were administered consistently across study groups (spiked daily) but how stock solutions were mixed was not reported			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured by GC-MS but were lower than nominal con- centrations.			
	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 suitable for a dose response (125, 250, 500, and 1000 ug/L DBP). 2 replicates containing 4 fish each.			
	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 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	Only 4 fish in duplicate were used.			

Continued on next page ...

HERO ID: 1639196 Table: 1 of 5

continued from previous page							
Study Citation:	Bhatia, H., H	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish					
	(Melanotaen	Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 days				
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; F	rish; Melanotaenia fluviatilis; Adult					
Health Outcome:	Hepatic/Live						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1639196						
Domain		Metric	Rating	Comments			
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 outcomes of interest.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for growth measurements were limited.			
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.			
	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 (one-way ANOVA).			
	Metric 22:	Reporting of Data	Medium	Some results were in text only, others were in table 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form wa	as added to account for the hepatic outcom	e of the HSI.				
Overall Quality Determination			High				

Study Citation:	Bhatia, H., I (Melanotaen	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.					
Exposure Route, Modia Pathy	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; I	Fish; <i>Melanotaenia fluviatilis</i> ; Adult					
Health Outcome:	Reproductiv	e/Teratogenic					
HERO ID:	1639196						
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	High	The test substance identity was verified by GC-MS.			
	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, and a positive vehicle control (0.001% methanol).			
	Metric 5:	Negative Control Response	Medium	No mortality in any treatments was reported. Some growth results were mentioned in the text as not significant.			
	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	High	The experimental system and methods for preparation of test media were described in adequate detail. Exposure was conducted in glass aquaria			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure was conducted in glass aquarta. Details of exposure administration were reported and exposures were administered consistently across study groups (spiked daily), but how stock solutions were mixed was not reported.			
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured by GC-MS but were lower than nominal con- centrations.			
	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 suitable for a dose response (125, 250, 500, and 1000 ug/L DBP). There were two replicates containing four fish each.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organisi	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 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	Only four fish in each duplicate were used.			

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 1639196 Table: 2 of 5

	continued from previous page				
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344. 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) Vertebrate; Fish; <i>Melanotaenia fluviatilis</i> ; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP) 1639196				
Domain		Metric	Rating	Comments	
	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 outcomes of interest.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for growth measurements were limited.	
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	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 (one-way ANOVA).	
	Metric 22:	Reporting of Data	Medium	Some results were in text only, while others were in table 2.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This form wa	as added to account for the reproductive ou	tcomes obtai	ned from histology and for the GSI.	
Overall Quality Determination		High			

Study Citation:	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish						
Duration	(Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.						
Duration: Evnosuro Douto	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, Modio Dothy	Aquatic (free	sinwater), water, Not determined by study	autions (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa Spacios Ago:	Vartabrata: E	Sish: Molanotaonia fluviatilis: Adult					
Hoolth Outcome	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1639196						
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	High	The test substance identity was verified by GC-MS.			
	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, and a positive vehicle control (0.001% methanol).			
	Metric 5:	Negative Control Response	Medium	No mortality in any treatments reported, some growth results were only in text as not significant.			
	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	High	The experimental system and methods for preparation of test media were described in			
	metric /:	Preparation	mgn	adequate detail. Exposure was conducted in glass aquaria.			
	Metric 8:	Consistency of Exposure	Medium	Details of exposure administration were reported and exposures were administered			
		Administration		consistently across study groups (spiked daily) but how stock solutions were mixed was not reported			
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured by GC-MS but were lower than nominal con- centrations.			
	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 spacing of exposure levels were suitable for a dose			
		Spacing of Exposure Levels		response (125, 250, 500, and 1000 ug/L DBP), 2 replicates containing 4 fish each.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
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	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	Low	Only 4 fish in duplicate were used.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.			
		Cont	inued on nex	t page			

HERO ID: 1639196 Table: 3 of 5

		conti	nued from p	revious page			
Study Citation:	Bhatia, H., I (Melanotaen	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.					
Duration:	Overall Dura	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:	Vertebrate; Fish; Melanotaenia fluviatilis; Adult						
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1639196						
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 Assessment	Low	Details regarding the execution of the study protocol for growth measurements were limited.			
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	High	Statistical methods were adequately described (one-way ANOVA).			
	Metric 22:	Reporting of Data	Medium	Some results were in text only, others were in table 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	High				

Study Citation:	Bhatia, H., K (Melanotaeni Overall Dura	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish (Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344. Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 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)				
Taxa, Species, Age:	Vertebrate; F	ish; <i>Melanotaenia fluviatilis</i> ; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	1639196					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT' 1			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#		
	Metric 2: Matria 2:	Test Substance Source	High	The test substance identity was verified by GC-MS.		
	Metric 5:	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	No mortality in any treatments were 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 test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	Medium	Details of exposure administration were reported and exposures were administered consistently across study groups (spiked daily) but how chemical was mixed was not		
		Administration		reported		
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but were lower than 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/	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 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	C C	were the same for control and exposed organisms		
	Metric 15:	Number of Organisms and	Low	Only 4 fish in duplicate were used		
		Replicates per Group				
Domain 5: Outcome As	sessment					
Zoman 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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HERO ID: 1639196 Table: 4 of 5

	continued from previous page					
Study Citation:	Bhatia, H., I	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish				
Duration:	(Melanotaen Overall Dura	(Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344. 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:						
Taxa, Species, Age:	Vertebrate; F	ish; Melanotaenia fluviatilis; Adult				
Health Outcome:	Mortality Dibutyl phth	alate (DRP)				
HERO ID:	1639196					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for mortality were limited		
		Assessment				
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	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	Low	Text reported no mortalities		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	None					
Overall Quality Determination		High				

Study Citation:	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish					
Duration: Exposure Route, Media. Path:	(Melanotaen 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: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mechanistic- Dibutyl phth 1639196	Vertebrate; Fish; <i>Melanotaenia fluviatilis</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Endocrine toxicity Dibutyl phthalate (DBP) 1639196				
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	High	The test substance identity was verified by GC-MS.		
	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	No mortality in any treatments reported, mechanistic results were reasonable		
	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	e	adequate detail		
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported and exposures were administered consistently across study groups (spiked daily) but how chemical was mixed was not reported		
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but were lower than 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 suitable for a dose response		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit		
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 and all pretreatment conditions		
	Metric 15:	Conditions Number of Organisms and	Low	were the same for control and exposed organisms Only 4 fish in duplicate were used		
		Replicates per Group				
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 outcomes of interest		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 1639196 Table: 5 of 5

continued from previous page						
Study Citation:	Bhatia, H., I	Bhatia, H., Kumar, A., Du, J., Chapman, J., Mclaughlin, M. J. (2013). Di-n-butyl phthalate causes antiestrogenic effects in female murray rainbowfish				
	(Melanotaen	(Melanotaenia fluviatilis). Environmental Toxicology and Chemistry 32(10):2335-2344.				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Koule, Modia Dath.	Aquatic (free	snwater); water; Not determined by study a	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	Vantahnata, T	ich Malanataonia Anniatilia Adult				
Taxa, Species, Age:	Machaniatia	Pisn; Metanoidenia juvianiis; Adult Biomoriyara (avrogumo and affect) Endoari	na taviaity			
Chamical	Dibutul abth	-biomarkers (exposure and effect)-Endocri	ne toxicity			
	1620106	alate (DBP)				
HERO ID;	1039190					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Details regarding the execution of the study protocol for mechanistic measurements were adequate		
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	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:	Biomarkers	were added to the mechanistic outcome to	account for v	ritellogenin levels.		
Overall Quali	Overall Quality Determination High					

Study Citation: Duration: Exposure Route, Media, Path:	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115. 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:	Vertebrate; F	Vertebrate; Fish; Melanotaenia fluviatilis; Males; Adult						
Health Outcome:	Hepatic/Live	r						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	2509291							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.				
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Sigma Aldrich, but it did not appear to be analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control as well as a solvent control.				
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Table 3 and were adequate for the out- come of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.				
Demein 2. Ernerum Ch	:::							
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	The system was reported to be semi-static with daily renewals, but the preparation of the test media was not reported				
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 35 x 18 x 24cm aquaria containing 5L of test solution with four fish per tank. All exposures were conducted at 21 C with a 16L:8D photoperiod. But measured concentrations did not appear to be close to nominal concentrations in some instances.				
	Metric 9:	Measurement of Test Substance	High	The test substance was measured using GC-MS.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported to be seven days, and this was adequate for a dose response.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four exposure levels plus a negative control and a solvent control. The spac- ing was appropriate to observe a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using a vehicle solvent, and the solvent control response was adequate.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from Aquarium Industries in Victoria, Australia. Fish were reported to be adult males.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were acclimated for 14d prior to the start of the test.				
Continued on next page								

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Bhatia, H., I male Murray Overall Dura Aquatic (fres Vertebrate; F Hepatic/Live Dibutyl phth	 Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115. 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) Vertebrate; Fish; <i>Melanotaenia fluviatilis</i>; Males; Adult Hepatic/Liver Dibutyl phthalate (DBP) 				
HERO ID:	2509291					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four fish per test chamber and two aquaria per treatment.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Fish were kept at 21C in artificial river water with a 16L:8D photoperiod. They were fed frozen brine shrimp at a rate of 4% (w/w) body weight.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-HSI.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Tissues were fixed after the seven-day exposure period. Assessment was discussed in detail in the paper.		
Domain 6: Confounding	y / Variable Co	ntrol				
	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 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 reported in the "Data Analysis" section.		
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response were reported in Table 3.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figures 3 and 4.		
Additional Comments:	This evaluati	on was for the HSI reported in Table 3. Th	e outcome se	elected was the hepatic outcome.		

Overall Quality Determination

High

Study Citation:	Bhatia, H., k	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanoteenia fluviatilic). Aquatic Toxicology 149(Elsevier):103, 115					
Duration:	Overall Dura	tion: 4 - 10 days; Exposure Duration: 4 - 1	10 days	logy 149(Elsevier).105-115.			
Exposure Route,	Aquatic (fres	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:	Vantalandar E	Vortabrata, Fish, Malanataonia Auviatilia, Malao, Adult					
Taxa, Species, Age: Health Outcome:	Vertebrate; Fish; <i>Melanotaenia fluviatilis</i> ; Males; Adult Reproductive/Teratogenic						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2509291						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.			
	Metric 2:	lest Substance Source	Low	The DBP was reported to be from Sigma Aldrich, but it did not appear to be analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control as well as a solvent control.			
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Figures 1-4 and were adequate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	The system was reported to be semi-static with daily renewals, but the preparation of the			
	Metric 8.	Preparation Consistency of Exposure	Medium	All exposures occurred in 35 x 18 x 24cm aquaria containing 5L of test solution with			
	Mettre 0.	Administration	Weddum	4 fish per tank. All exposures were conducted at 21 C with a 16L:8D photoperiod. But measured concentrations did not appear to be close to nominal concentrations in some instances.			
	Metric 9:	Measurement of Test Substance	High	The test substance was measured using GC-MS.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported to be 7 days, and this was adequate for a dose response.			
	Metric 11:	Number of Exposure Groups/	High	There were 4 exposure levels plus a negative control and a solvent control. The spacing			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Study authors reported using a vehicle solvent, and the solvent control response was adequate.			
Domain 4 [,] Test Organisi	n						
20man 1. rest organisi	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from Aquarium Industries in Victoria, Australia. Fish were reported to be adult males.			
	Metric 14:	Acclimatization and Pretreatment	High	The fish were acclimated for 14d prior to the start of the test.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 2 aquaria per treatment.			
		Replicates per Group					
		Conti	inued on nex	t page			

HERO ID: 2509291 Table: 2 of 5

		conti	nued from p	revious page			
Study Citation:	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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	Fish; Melanotaenia fluviatilis; Males; Adult	t				
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2509291						
Domain	2307271	Metric	Rating	Comments			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were kept at 21C in artificial river water with a 16L:8D photoperiod. They were fed frozen brine shrimp at a rate of 4% (w/w) body weight.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-histological changes in the testes.			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups. Tissues were fixed after the 7 day exposure period. Assessment was discussed in detail in the paper.			
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.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Data Analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response were reported in Figures 1-4 and were adequate.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Fig- ures 3 and 4			

Overall Quality Determination

High

Study Citation:	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult							
Duration	male Murray	male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115. Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days						
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:	riquite (ires	in all all of the second start and a second start and a second start and a second start and second start and se	uuiors (1.e., 1					
Taxa, Species, Age:	Vertebrate; F	ish; <i>Melanotaenia fluviatilis</i> ; Males; Adult						
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)						
Chemical:	Dibutyl phtha	Dibutyl phthalate (DBP)						
HERO ID:	2509291	/509291						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	e .							
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.				
	Metric 2:	lest Substance Source	Low	The DBP was reported to be from Sigma Aldrich, but it did not appear to be analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control as well as a solvent control.				
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Figures 5-7 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.				
	, . <u>.</u> .							
Domain 3: Exposure Cha	Matria 7	Europin antal System/Test Madia	Low	The surface and state he saw is the surface of the surface of the surface of the				
	Metric 7:	Preparation	LOW	test media was not reported				
	Metric 8:	Consistency of Exposure	Medium	All exposures occurred in 35 x 18 x 24cm aquaria containing 5L of test solution with				
		Administration		4 fish per tank. All exposures were conducted at 21 C with a 16L:8D photoperiod. But measured concentrations did not appear to be close to nominal concentrations in some instances.				
	Metric 9:	Measurement of Test Substance	High	The test substance was measured using GC-MS.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported to be 7 days, and this was adequate for a dose				
	Metric 11.	Number of Exposure Groups/	High	response. There were 4 exposure levels plus a negative control and a solvent control. The spacing				
	mune 11.	Spacing of Exposure Levels	mgn	was appropriate to observe a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using a vehicle solvent, and the solvent control response was adequate.				
Domain 4: Test Organisi	n Matri - 12	Test Organism Characteristics	IE-b					
	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from Aquarium Industries in Victoria, Australia. Fish were reported to be adult males.				
	Metric 14:	Acclimatization and Pretreatment	High	The fish were acclimated for 14d prior to the start of the test.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 2 aquaria per treatment.				
		Replicates per Group						
	Continued on next page							

HERO ID: 2509291 Table: 3 of 5

continued from previous page						
Study Citation:	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115					
Duration:	Overall Duration: 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:	Vertebrate; F	Fish; Melanotaenia fluviatilis; Males; Adul	ţ			
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2509291					
Domain	Metric Rating Comments					
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	High	Fish were kept at 21C in artificial river water with a 16L:8D photoperiod. They were fed frozen brine shrimp at a rate of 4% (w/w) body weight.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–gene expression/transcription.		
	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	e	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	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Data Analysis" section.		
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response were reported in Figures 5-7 and were adequate.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figures 5-7		

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path:	Bhatia, H., k male Murray Overall Dura Aquatic (fres	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115. 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:	Vertebrate; Fish; Melanotaenia fluviatilis; Males; Adult					
Health Outcome:	Development	t/Growth				
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	2509291					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.		
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Sigma Aldrich, but it did not appear to be analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP 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 as well as a solvent control.		
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Table 3 and was adequate for the out- come of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.		
Domain 3: Exposure Cha	aracterization	Experimental System/Test Madia	Low	The system was compared to be carried to be in with daily compared by the propagation of the		
	Metric 7:	Preparation	Low	test media was not reported.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 35 x 18 x 24cm aquaria containing 5L of test solution with 4 fish per tank. All exposures were conducted at 21 C with a 16L:8D photoperiod. But measured concentrations did not appear to be close to nominal concentrations in some instances.		
	Metric 9:	Measurement of Test Substance	High	The test substance was measured using GC-MS.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported to be 7 days, and this was adequate for a dose response.		
	Metric 11:	Number of Exposure Groups/	High	There were 4 exposure levels plus a negative control and a solvent control. The spacing was appropriate to observe a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using a vehicle solvent, and the solvent control response was adequate.		
Domain 4: Test Organist	n					
_ main in rost organish	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from Aquarium Industries in Victoria, Australia. Fish were reported to be adult males.		
	Metric 14:	Acclimatization and Pretreatment	High	The fish were acclimated for 14d prior to the start of the test.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 2 aquaria per treatment.		
		Replicates per Group				
		Conti	nued on nex	t page		

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Dibutyl Phthalate

		conti	nued from p	previous page			
Study Citation:	Bhatia, H., I male Murray	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	l 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:	Vertebrate; F	Fish; Melanotaenia fluviatilis; Males; Adult	t				
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2509291						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were kept at 21C in artificial river water with a 16L:8D photoperiod. They were fed frozen brine shrimp at a rate of 4% (w/w) body weight.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-body weight and organ weight changes.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Body weights and lengths were taken at the end of the 7 days, and organs weights were also taken at this time to calculate GSI and HSI and CF.			
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.			
Domain 7: Data Present	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Data Analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response were reported in Table 3 and were adequate.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 3.			
Additional Comments:	This evaluati the outcome	ion was on the effects of DBP on organ we of interest.	ights and boo	dy weight and length of the Murray rainbow fish. Development/growth was selected as			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Bhatia, H., H male Murray Overall Dura Aquatic (fres	Kumar, A., Ogino, Y., Gregg, A., Chapma rainbowfish (Melanotaenia fluviatilis). Ad ation: 4 - 10 days; Exposure Duration: 4 - shwater): Water: Not determined by study i	n, J., Mclaug quatic Toxico 10 days 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>Melanotaenia fluviatilis</i> ; Males; Adult Mortality Dibutyl phthalate (DBP) 2509291					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.		
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Sigma Aldrich, but it did not appear to be analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control as well as a solvent control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in section 3.3.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The system was reported to be semi-static with daily renewals, but the preparation of the test media was not reported.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 35 x 18 x 24cm aquaria containing 5L of test solution with 4 fish per tank. All exposures were conducted at 21 C with a 16L:8D photoperiod. But measured concentrations did not appear to be close to nominal concentrations in some instances.		
	Metric 9:	Measurement of Test Substance	High	The test substance was measured using GC-MS.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the exposure was reported to be 7 days, and this was adequate for a dose response.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 4 exposure levels plus a negative control and a solvent control. The spacing was appropriate to observe a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported using a vehicle solvent, and the solvent control response was adequate.		
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	High	The fish were reported to be from Aquarium Industries in Victoria, Australia. Fish were reported to be adult males.		
	Metric 14:	Acclimatization and Pretreatment	High	The fish were acclimated for 14d prior to the start of the test.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 2 aquaria per treatment.		
		Replicates per Group				

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

		conul	nuea from p	revious page			
Study Citation:	Bhatia, H., I male Murray	Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., Mclaughlin, M. J., Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (Melanotaenia fluviatilis). Aquatic Toxicology 149(Elsevier):103-115.					
Duration:	Overall Duration: 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:	Vertebrate; F	Fish; Melanotaenia fluviatilis; Males; Adult	t				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2509291						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were kept at 21C in artificial river water with a 16L:8D photoperiod. They were fed frozen brine shrimp at a rate of 4% (w/w) body weight.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome 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: 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.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Data Analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response were reported in Section 3.3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figures 3 and 4.			

Additional Comments: Section 3.3 reported no mortality among the fish. This form was created for that outcome.

Overall Quality Determination	High	

Study Citation:	Bhatia, H., H development	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816.						
Duration: Exposure Route,	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)						
Media, Path:	Vertebrate: Fish: Melanotaenia fluviatilis: Iuvenile							
Health Outcome:	Mortality	Mortality						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	2816886							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.				
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich in Australia, but it was not reported if it was analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	It was reported a concurrent negative "water" control was used as well as a solvent con- trol.				
	Metric 5:	Negative Control Response	Low	The response of the negative control was not reported for mortality.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into 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	Low	Limited details were provided on the preparation of the stock solution and the test con- centrations.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were conducted in 1L for the first 30d and then in 2L for the rest of the study with four fish per glass beaker. Temperature and photoperiod remained the same for the duration of the study.				
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	Exposures were for up to 90 days with sampling at 30, 60, and 90 days. This was ade- quate to observe a response.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only three exposure levels, which is lower than normal, but still showed a response.				
	Metric 12:	Testing at or Below Solubility Limit	High	It was reported that DMSO was used as a vehicle solvent. The solvent control response was appropriate.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	Organisms were from the Aquarium Industries in Victoria, Australia. Juvenile fish were used for this study.				
	Metric 14:	Acclimatization and Pretreatment	High	Fish were acclimated to test conditions for two weeks prior to the start of this study.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were four fish per test chamber and four replicates per treatment per time interval.				
		Replicates per Group						
Continued on next page								

Environmental Hazard Evaluation

HERO ID: 2816886 Table: 1 of 4

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816. 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; Melanotaenia fluviatilis; Juvenile					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816886						
Domain	Metric Rating Comments						
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be kept at a loading rate of 0.5g/L at a temperature of 23C with a 16L:8D photoperiod. They were fed artemia daily at 4% (w/w).			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome 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. The fish were sampled at each time interval and were measured and weighed at this point.			
Domain 6: Confounding	/ Variable Cor	ntrol					
C	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	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 Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analyses" section of the paper.			
	Metric 22:	Reporting of Data	Low	Results for mortality were reported in the text only.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Fig- ure 1.			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816. 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>Melanotaenia fluviatilis</i> ; Juvenile Development/Growth Dibutyl phthalate (DBP) 2816886					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce		0			
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.		
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich in Australia, but it was not reported if it was analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.		
Domain 2: Test Design	Metric 4:	Negative Controls	High	It was reported a concurrent negative "water" control was used as well as a solvent con-		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- comes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the stock solution and the test con- centrations.		
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were conducted in 1L for the first 30d and then in 2L for the rest of the study with 4 fish per glass beaker. Temperature and photoperiod remained the same for the duration of the study.		
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	Exposures were for up to 90 days with sampling at 30, 60, and 90 days. This was ade- quate to observe a response.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only 3 exposure levels, which is lower than normal, but still showed a re- sponse.		
	Metric 12:	Testing at or Below Solubility Limit	High	It was reported that DMSO was used as a vehicle solvent. The solvent control response was appropriate.		
Domain 4. Tast Organise	m					
Domani 4. 16st Organisi	Metric 13:	Test Organism Characteristics	High	Organisms were from the Aquarium Industries in Victoria, Australia. Juvenile fish were used for this study.		
	Metric 14:	Acclimatization and Pretreatment	High	Fish were acclimated to test conditions for 2 weeks prior to the start of this study.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 4 replicates per treatment per time interval.		
		Replicates per Group				

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Environmental Hazard Evaluation

HERO ID: 2816886 Table: 2 of 4

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Study Citation:	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murrav rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816.			
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; Melanotaenia fluviatilis; Juvenile			
Health Outcome:	Development/Growth			
Chemical:	Dibutyl phthalate (DBP)			
HERO ID:	2816886			
Domain		Metric	Rating	Comments
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be kept at a loading rate of $0.5g/L$ at a temperature of 23C with a 16L:8D photoperiod. They were fed artemia daily at 4% (w/w).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-the growth of the fish in terms of length and weight.
	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 sampled at each time interval and were measured and weighed at this point.
Domain 6: Confounding	g / Variable Co	ntrol		
·	Metric 19:	Confounding Variables in Test	Medium	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 Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analyses" section of the paper.
	Metric 22:	Reporting of Data	High	The control response and the exposure response were adequate and were reported in Figure 1.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 1.
Additional Comments:	This portion of the evaluation was on the effect of DBP on th of interest.			ght and length of M. fluviatilis. Development and growth was selected as the outcome
Overall Quali	ty Deterr	nination	High	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bhatia, H., F development Overall Dura Aquatic (fres Vertebrate; F Reproductiva Dibutyl phth 2816886	Kumar, A., Chapman, J. C., Mclaughlin, in juvenile Murray rainbowfish (Melanota tion: > 21 days; Exposure Duration: > 2 hwater); Water; Not determined by study ish; <i>Melanotaenia fluviatilis</i> ; Juvenile e/Teratogenic alate (DBP)	M. J. (2015). aenia fluviatil 1 days authors (i.e.,	Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad is). Journal of Applied Toxicology 35(7):806-816. chemical of interest in exposure water, but unable to determine exact uptake route)
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Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce	Wettle	Rating	connients
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich in Australia, but it was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.
Domain 2: Test Design	Metric 4:	Negative Controls	High	It was reported a concurrent negative "water" control was used as well as a solvent con- trol.
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 2 and 3 and was adequate for the outcomes of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the stock solution and the test con- centrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were conducted in 1L for the first 30d and then in 2L for the rest of the study with 4 fish per glass beaker. Temperature and photoperiod remained the same for the duration of the study.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.
	Metric 10:	Concentration Exposure Duration and Frequency	High	Exposures were for up to 90 days with sampling at 30, 60, and 90 days. This was ade- quate to observe a response.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only 3 exposure levels, which is lower than normal, but still showed a response.
	Metric 12:	Testing at or Below Solubility Limit	High	It was reported that DMSO was used as a vehicle solvent. The solvent control response was appropriate.
Domain 4: Test Organisi	m			
	Metric 13:	Test Organism Characteristics	High	Organisms were from the Aquarium Industries in Victoria, Australia. Juvenile fish were used for this study.
	Metric 14:	Acclimatization and Pretreatment	High	Fish were acclimated to test conditions for 2 weeks prior to the start of this study.
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 4 replicates per treatment per time interval.
		Replicates per Group		

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 2816886 Table: 3 of 4

		conti	nued from p	revious page			
Study Citation:	Bhatia, H., I development	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816.					
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:	Aquate (neshwater), water, Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake foure)						
Taxa. Species. Age:	Vertebrate: Fish: Melanotaenia fluviatilis: Iuvenile						
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	2816886						
Domain	Metric Rating Comments						
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be kept at a loading rate of $0.5g/L$ at a temperature of 23C with a 16L:8D photoperiod. They were fed artemia daily at 4% (w/w).			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-histopathological changes in the gonads.			
	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 sampled at each time interval and were fixed investigation of gonad development by whole body sectioning. Parameters were set for staging the gonads.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	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 Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analyses" section of the paper.			
	Metric 22:	Reporting of Data	High	The control response and the exposure response were adequate and were reported in Figures 2-4.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	: This portion of the evaluation was on the effect of DBP on the gonads of the M. fluviatilis. Histopathology was used to assess the organs. Rep was selected as the outcome of interest.						
Overall Qualit	ty Deterr	nination	High				

Study Citation:	Bhatia, H., F development Overall Dura	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816.					
Exposure Route, Media, Path:	Aquatic (fres	hwater); 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; Melanotaenia fluviatilis; Juvenile					
Health Outcome:	Mechanistic-	Endocrine toxicity					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816886						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		TT: 1				
	Metric 1: Matria 2:	Test Substance Identity	High	The test substance was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	and source was reported to be Sigma Aldrich in Australia, but it was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.			
Domain 2. Tast Dasign							
Domani 2. Test Design	Metric 4:	Negative Controls	High	It was reported a concurrent negative "water" control was used as well as a solvent con-			
				trol.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 5 and was adequate for the out- comes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 2. Euroques Ch	anastanization						
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media	Low	Limited datails were provided on the preparation of the stock solution and the test con			
	Wiettie 7.	Preparation	LOW	centrations.			
	Metric 8:	Consistency of Exposure	Medium	Exposures were conducted in 1L for the first 30d and then in 2L for the rest of the study			
		Administration		with 4 fish per glass beaker. Temperature and photoperiod remained the same for the duration of the study.			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using GC-MS.			
	Metric 10:	Exposure Duration and Frequency	High	Exposures were for up to 90 days with sampling at 30, 60, and 90 days. This was ade- quate to observe a response.			
	Metric 11:	Number of Exposure Groups/	Medium	There were only 3 exposure levels, which is lower than normal, but still showed a re-			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	sponse. It was reported that DMSO was used as a vehicle solvent. The solvent control response			
	moune 12.	Testing at of Delow Solubility Emile	mgn	was appropriate.			
Domain 4: Test Organisi	m Metric 13.	Test Organism Characteristics	High	Organisms were from the Aquarium Industries in Victoria, Australia, Invanila fish ware			
	mente 15.	rest Organism Characteristics	mgn	used for this study.			
	Metric 14:	Acclimatization and Pretreatment	High	Fish were acclimated to test conditions for 2 weeks prior to the start of this study.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 4 fish per test chamber and 4 replicates per treatment per time interval.			
		Replicates per Group					
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 2816886 Table: 4 of 4

		conti	nued from p	revious page			
Study Citation:	Bhatia, H., I development	Bhatia, H., Kumar, A., Chapman, J. C., Mclaughlin, M. J. (2015). Long-term exposures to di-n-butyl phthalate inhibit body growth and impair gonad development in juvenile Murray rainbowfish (Melanotaenia fluviatilis). Journal of Applied Toxicology 35(7):806-816.					
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; <i>Melanotaenia fluviatilis</i> ; Juvenile						
Health Outcome:	Mechanistic	-Endocrine toxicity					
Unemical:	Dibutyl phth	ialate (DBP)					
HERO ID:	2816886						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be kept at a loading rate of 0.5 g/L at a temperature of 23C with a 16L:8D photoperiod. They were fed artemia daily at 4% (w/w).			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-hormone levels.			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups. The fish were sampled at each time interval and were snap frozen until enzyme analysis. EIA kits were used to assess the hormone levels.			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	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 Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analyses" section of the paper.			
	Metric 22:	Reporting of Data	High	The control response and the exposure response were adequate and were reported in Figure 5.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 5.			
Additional Comments:	This portion outcome of i	of the evaluation was on the effect of D interest.Mortality was also mentioned in th	BP on hormo	one levels in M. fluviatilis. The mechanistic endocrine outcome was selected as the vas not quantified anywhere, so an evaluation was not completed for it.			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media. Path:	EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. 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:	Vertebrate; F Mortality	ish; Oncorhynchus mykiss; Juvenile				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	65/1362					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce		Ŧ			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.		
	Metric 2:	Test Substance Source	Hıgh	The source of the DBP was reported to be the Chemical Manufacturers Association in Washington DC, and was analytically verified by GC-MS.		
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.		
	Metric 5:	Negative Control Response	Low	The negative control for the preliminary test was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how juvenile fish were allocated into study groups in the preliminary test.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Low	All exposures for the preliminary test were conducted for 13 days. The test chambers for this portion of the study were not described. Little other information was provided regarding test conditions for the preliminary study.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if test concentrations were measured in the preliminary study.		
	Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 13 days. This was adequate for a preliminary test.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups as well as a negative control and a solvent control. This is typical for testing, and spacing was appropriate to observe a response.		
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.		
Domain 4: Test Organisi	n					
	Metric 13:	Test Organism Characteristics	High	The trout were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.		
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation was not reported for the preliminary test.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of fish per replicate and the number of replicates were not reported for the preliminary study.		

Domain 5: Outcome Assessment

Continued on next page ...

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Dibutyl Phthalate

continued from previous page								
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	EnviroSyster Overall Dura Aquatic (fres Vertebrate; F Mortality Dibutyl phtha 6571362	EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. 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>Oncorhynchus mykiss</i> ; Juvenile Mortality Dibutyl phthalate (DBP) 6571362						
Domain		Metric	Rating	Comments				
	Metric 16: Metric 17:	Adequacy of Test Conditions Outcome Assessment Methodology	Low High	Test conditions were not reported for the preliminary study. The outcome assessment methodology addressed or reported the intended outcomes of interest–fish survival in the terms of LC50 values for 96h and 13d.				
	Metric 18:	Consistency of Outcome Assessment	Low	Minimal details were provided regarding outcome assessment for the preliminary 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.				
Domain 7: Data Presenta	ation and Anal	ysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately for the preliminary study.				
	Metric 22:	Reporting of Data	Low	Data were only reported in the text for the preliminary test.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported for the preliminary study.				
Additional Comments:	This evaluati preliminary t	This evaluation is for the preliminary test conducted with juvenile rainbow trout. 96h and 13d LC50 values were reported. Little information regarding preliminary test protocol and procedures was reported. Mortality was the outcome of interest.						

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Study Citation: Duration: Exposure Route, Media, Path:	EnviroSyster Overall Dura Aquatic (fres	n, (1991). Early life-stage toxicity of di- tion: > 21 days; Exposure Duration: > 22 shwater); Water; Not determined by study	butyl phthala l days authors (i.e., o	te (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. 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	t/Growth		
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	6571362			
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 DBP was reported to be the Chemical Manufacturers Association in Washington DC. It was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response and the vehicle control response were reported in Tables 4, 5, and in B3-B7.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly distributed to each exposure group.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	Little information was provided on the preparation of the test concentrations
	Weate 7.	Preparation	Low	Ende mornation was provided on the preparation of the test concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were for 99 days unless all the organisms in the test concentration had died prior to the end of the study. All the tests were conducted in 14 x 53 x 25cm with 15L of test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	The test concentrations were measured by two methods, a radiochemical analytical method and via GC/MS.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 99 days. This was adequate to observe a response across study groups.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups as well as a negative control and a solvent control. This is typical for testing, and spacing was appropriate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.
Domain 4: Tast Organia	~			
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	The embryos and sperm were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.
	Metric 14:	Acclimatization and Pretreatment	Medium	Acclimation was reported, but the duration was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 30 embryos per test chamber with two replicates. Two replicates is less than is typical, thus the low ranking.

Continued on next page ...

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Environmental Hazard Evaluation

HERO ID: 6571362 Table: 1 of 2

		contin	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. 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; Oncorhynchus mykiss; Embryo					
Health Outcome:	Developmen Dibutyl phth	Development/Growth					
HERO ID:	6571362						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Embryos were held at 10C for the first 6 weeks and then the temperature was gradually increased to 12.5C over week 7 to the end of the study. Embryos were kept in the dark until day 43, at the start of swim-up, and then kept at a photoperiod of 14L:10D. Fry were fed starter mash and live brine shrimp.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–lengths and weights of larval fish, as well as any other sublethal effects.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Caliper lengths and photographic lengths were taken at the end of the study, and wet weights and dry weights were both taken at the end of the study.			
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 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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "statistical analysis" section of the report.			
	Metric 22:	Reporting of Data	High	Body weights and lengths were provided as well as other sublethal effects were reported in Tables 4, 5, and B3-B7.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.			
Additional Comments:	This portion outcome of i	of the evaluation was on the effect of DBP nterest.	on fish lengt	h and width, as well as other sublethal effect. Development/growth was selected as the			
Overall Qualit	y Detern	nination	High				

Dibutyl Phthalate

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HERO ID: 6571362 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media. Path:	EnviroSyster Overall Dura Aquatic (free	m, (1991). Early life-stage toxicity of di- ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	-butyl phthala 1 days authors (i.e.,	te (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. 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:	Dibutyl phth	alate (DBP)		
HERO ID:	6571362			
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	High	The source of the DBP was reported to be the Chemical Manufacturers Association in Washington DC, and was analytically verified by GC-MS.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response and the vehicle control response were reported in Table 3 and in Tables B1 and B2.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly distributed to each exposure group.
Damain 2. Enna ann Ch				
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Law	Time information and in the second in the second in the second second second second second second second second
	Metric 7:	Experimental System/Test Media	LOW	Little information was provided on the preparation of the test concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were for 99 days unless all the organisms in the test concentration had died prior to the end of the study. All the tests were conducted in $14 \times 53 \times 25$ cm with
	Metric 9:	Measurement of Test Substance	High	The test concentrations were measured by two methods, a radiochemical analytical
	Metric 10:	Concentration Exposure Duration and Frequency	High	The test duration was reported to be 99 days. This was adequate to observe a response
	Metric 11:	Number of Exposure Groups/	High	There were five exposure groups as well as a negative control and a solvent control. This is tunical for testing, and spacing was appropriate to observe a response
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.
Domain 4: Test Organisi	n			
	Metric 13:	Test Organism Characteristics	High	The embryos and sperm were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.
	Metric 14:	Acclimatization and Pretreatment	Medium	Acclimation was reported, but the duration was not reported.
	Metric 15:	Conditions Number of Organisms and	Low	There were 30 embryos per test chamber with two replicates. Two replicates is less than
		Replicates per Group		is typical, mus me low fallking.

Domain 5: Outcome Assessment

Continued on next page ...

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Dibutyl Phthalate

Study Citation: EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. **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; Oncorhynchus mykiss; Embryo **Health Outcome:** Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 6571362 Domain Metric Comments Rating Metric 16: Adequacy of Test Conditions High Embryos were held at 10C for the first six weeks and then the temperature was gradually increased to 12.5C over week seven to the end of the study. Embryos were kept in the dark until day 43, at the start of swim-up, and then kept at a photoperiod of 14L:10D. Fry were fed starter mash and live brine shrimp. Metric 17: Outcome Assessment Methodology High The outcome assessment methodology addressed or reported the intended outcomes of interest-embryo/larval survival and percent hatch. Metric 18: Consistency of Outcome High Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Embryos and larvae were observed daily for mortali-Assessment ties. 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 or other factors that could influence the outcome assessment. Outcomes Unrelated to Exposure Metric 20: 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 in the "statistical analysis" section of the report. Metric 22: Reporting of Data High Mortality data for the control responses and the exposure responses were reported in Tables 3, B1, and B2 and were adequate for the outcomes of interest.

Additional Comments: This portion of the evaluation was on the effect of DBP on embryo survival, percent hatch, and larval survival at various points in the 99 day study. Mortality was selected as the outcome of interest.

Study authors did not report any unexpected outcomes.

High

Overall Quality Determination

Metric 23:

Explanation of Unexpected Outcomes

High

Study Citation:	Rhodes, J. E	., Adams, W. J., Biddinger, G. R., Robill	ard, K. A., C	Forsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and				
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	Sish; Oncorhynchus mykiss; Embryo						
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	680120							
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	Test substance purity was reported as >95%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control and solvent control				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.				
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
2 011111 01 2.1900110 01	Metric 7:	Experimental System/Test Media Prenaration	Medium	The experimental system and methods for preparation of test media were described 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/ Spacing of Exposure Levels	High	The number of exposure groups and 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: Tast Organise								
Domain 4: Test Organisi	III Matria 12.	Test Organism Characteristics	Ulah	The test organisms were adequately described and were obtained from a reliable				
	Metric 13:	A solid stight in and Destruction of the	nign L	The test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Conditions	Low	The study did not report whether test organisms were acclimatized.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers of test organisms was adequate, but only two replicates were reported.				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to maintenance of organism health.				
		Cont	inued on nex	t page				

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HERO ID: 680120 Table: 1 of 2

		contir	ued from p	previous page			
Study Citation:	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: > 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	t/Growth					
Chemical:	Dibutyl phthalate (DBP)						
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	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	0				
	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. Dua Present	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Reporting of Data	Low	Data were only reported for the most sensitive outcomes			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
		~ *		-			
Additional Comments:	None						
	D-4-	· · · · · · · · · · · · · · · · · · ·	TT!1				
Overall Quality	iy Deterr	ninauon	High				

Study Citation:	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: Exposure Route, Media, Path:	rainbow trou 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: Health Outcome:	Vertebrate; F Mortality	ish; Oncorhynchus mykiss; Embryo				
Chemical: HERO ID:	Dibutyl phth 680120	alate (DBP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	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 >95%		
Domain 2: Test Design						
6	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control and solvent control		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group		
Domain 3: Exposure Ch	oracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Medium	The experimental system and methods for preparation of test media were described in		
	Wieute 7.	Preparation	Wiedium	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	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 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 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	Low	The study did not report whether test organisms were acclimatized		
	Metric 15:	Conditions Number of Organisms and	Low	The numbers of test organisms was adequate but only two replicates were reported		
		Replicates per Group				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to 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		
	Continued on next page					

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HERO ID: 680120 Table: 2 of 2

continued from previous 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 ainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976.					
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; F	Fish; Oncorhynchus mykiss; Embryo					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	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	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 22:	Reporting of Data	Low	Data were only reported for the most sensitive outcomes			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	None						
Overall Qualit	Overall Ouality Determination High						

Study Citation:	Bionomics,,	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report)				
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:	Vertebrate; H	Fish; Oncorhynchus mykiss (Salmo gairdne	eri); Adult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5530771					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT: 1			
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.		
	Metric 2:	Test Substance Source	High	11 and 18 December 1981.		
	Metric 3:	Test Substance Purity	High	The test substance was reported as "100% active ingredient." 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 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	High	Test concentrations were measured during the experiment. Endpoints were based on measured concentrations.		
	Metric 10:	Exposure Duration and Frequency	High	This was a 96-h acute exposure.		
	Metric 11:	Number of Exposure Groups/	High	The range of concentrations and number of groups were acceptable to determine LC50		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	values. 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 concentra- tions were used to calculate LC50s.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	Fish were obtained from commercial suppliers in Maryland and Montana. Lot numbers were given.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatization was for a minimum of 14 days in holding tanks.		
Continued on next page						

HERO ID: 5530771 Table: 1 of 1

		conti	nued from p	revious page		
Study Citation:	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					
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:	1					
Taxa, Species, Age:	Vertebrate; F	Fish; Oncorhynchus mykiss (Salmo gairdne	eri); Adult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5530771					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten fish per group and two replicates per concentration.		
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for husbandry of rainbow trout.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome was assessed appropriately (mortality).		
	Metric 18:	Consistency of Outcome	High	The outcome was assessed consistently among study groups.		
Domain 6: Confoundin	9 / 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 Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	The LC50 was calculated by a customized computer program using moving average angle analysis, probit analysis, or binomial probability. Details of the program were not reported.		
	Metric 22:	Reporting of Data	High	Data were reported for all groups.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					

Study Citation: Duration: Exposure Route	Erkmen, B., Nile tilapia (Overall Dura	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. 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 interact in exposure water, but unable to determine exact untake route).					
Media, Path:	Aquate (neshwater), water, not determined by study autions (i.e., enclinear of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate: F	ish: Oreochromis niloticus: Juvenile					
Health Outcome:	Mortality	· , - · · · · · · · · · · · · · · · · ·					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3974179						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.			
	Metric 3:	Test Substance Purity	Low	The purity/grade was not reported.			
Domain 2: Test Design	Matria 4.	Nagative Canterla	TT: -1-				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control.			
	Metric 5:	Regarized Allocation	High	I ne negative control response was reported in the text.			
	Metric 6:	Randomized Allocation	Low	It was not reported now the fish were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test media and test concentration was not reported. Exposure was conducted in 60L glass aquaria.			
	Metric 8:	Consistency of Exposure	High	All exposures were for either 24 or 96h in 60L tanks at 23C with 10 fish per tank.			
	Metric 9:	Administration Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be up to 96h with sampling done at 24h and 96h. This was appropriate for the outcome of interest.			
	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 the oxidative stress effect of one sublethal concentration of DBP on the fish.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentration was below the water solubility limit.			
Domain 4: Test Organis	n						
	Metric 13:	Test Organism Characteristics	High	The tilapia were from the Cukurova University Freshwater Fish Production Station in Adana, Turkey and were juveniles.			
	Metric 14:	Acclimatization and Pretreatment	High	The tilapia were acclimated for 15d prior to the start of the study.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 fish per test chamber and 2 tanks per treatment per exposure duration.			
		Replicates per Group					

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 3974179 Table: 1 of 5

continued from previous page						
Study Citation:	Erkmen, B., Nile tilapia (Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 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:	Vertebrate; F	Fish; Oreochromis niloticus; Juvenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3974179					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	The tilapia were kept in 60L tanks with 10 fish per tank at 23C with a 16L:8D photope- riod. They were fed commercial trout food at 2% their body weight.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome 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	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.		
	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 methods were described under the "Data Analysis" section of the paper.		
	Metric 22:	Reporting of Data	Low	Control and exposure responses were reported in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion	of the evaluation was on the effect of DBP	mortality.			
Overall Qualit	ty Detern	nination	High			

Study Citation:	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species						
Duration: Exposure Route,	Overall Dura Aquatic (fres	ation: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study a	in: 0 - 4 days authors (i.e.,	(0-96h) 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 Respiratory Dibutyl phth 3974179	Vertebrate; Fish; <i>Oreochromis niloticus</i> ; Juvenile Respiratory Dibutyl phthalate (DBP) 3974179					
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.			
	Metric 3:	Test Substance Purity	Low	The purity/grade was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3: Exposure Cha	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test media and test concentration was not reported. Exposure was conducted in 60L glass aquaria.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for either 24 or 96h in 60L tanks at 23C with 10 fish per tank.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be up to 96h with sampling done at 24h and 96h. This was appropriate for the outcome of interest.			
	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 the histological effect of one sublethal concentration of DBP on the fish.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentration was below the water solubility limit.			
Demain 4: Test O							
Domain 4: Test Organisi	n Metric 13:	Test Organism Characteristics	High	The tilapia were from the Cukurova University Freshwater Fish Production Station in			
	Metric 14:	Acclimatization and Pretreatment	High	The tilapia were acclimated for 15d prior to the start of the study.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 10 fish per test chamber and 2 tanks per treatment per exposure duration.			
Domain 5: Outcome Acc	ecoment						
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	The tilapia were kept in 60L tanks with 10 fish per tank at 23C with a 16L:8D photope- riod. They were fed commercial trout food at 2% their body weight.			
		Conti	nued on nex	t page			

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Dibutyl Phthalate

		conti	inued from p	previous page		
Study Citation: Duration: Exposure Route, Media, Path:	Erkmen, B., Nile tilapia (Overall Dura Aquatic (frea	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. 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	Vertebrate; Fish; Oreochromis niloticus; Juvenile				
Health Outcome:	Respiratory					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3974179					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in gill histology.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Liver and gill tissue was fixed at the end of the study and examined via microscope for histological changes.		
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.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described under the "Data Analysis" section of the paper.		
	Metric 22:	Reporting of Data	High	Control and exposure responses are reported in Figure 1 and were adequate for the out- come of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion	of the evaluation was on the effect of DBI	P on the gills.	Histopathology was performed, so the respiratory outcome was chosen.		

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. 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:	1 、						
Taxa, Species, Age:	Vertebrate; F	ish; Oreochromis niloticus; Juvenile					
Health Outcome:	Mechanistic-	Oxidative stress (including redox biology)	-Immune/He	matological			
Chemical:	Dibutyl phtha	alate (DBP)					
HERO ID:	3974179						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.			
	Metric 3:	Test Substance Purity	Low	The purity/grade was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 for MDA and GSH and was ade- quate for the outcome of interest. HCT control responses were presented in the text.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3: Exposure Cha	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test media and test concentration was not reported. Exposure was conducted in 60L glass aquaria.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for either 24 or 96h in 60L tanks at 23C with 10 fish per tank.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The study duration was reported to be up to 96h with sampling done at 24h and 96h. This was appropriate for the outcome of interest.			
	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 the oxidative stress effect of one sublethal concentration of DBP on the fish.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentration was below the water solubility limit.			
Domain 4. Test Organise	n						
Domain 1. 1050 Organisi	Metric 13:	Test Organism Characteristics	High	The tilapia were from the Cukurova University Freshwater Fish Production Station in Adana, Turkey and were juveniles.			
	Metric 14:	Acclimatization and Pretreatment	High	The tilapia were acclimated for 15d prior to the start of the study.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten fish per test chamber and two tanks per treatment per exposure duration.			
Domain 5: Outcome Age	ecoment						
Domani J. Outcome ASS	Metric 16:	Adequacy of Test Conditions	High	The tilapia were kept in 60L tanks with 10 fish per tank at 23C with a 16L:8D photope- riod. They were fed commercial trout food at 2% their body weight.			
		Conti	nued on nex	t page			

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Environmental Hazard Evaluation

HERO ID: 3974179 Table: 3 of 5

		conti	nued from p	revious page			
Study Citation:	Erkmen, B., Nile tilapia (Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilania (Oreachromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685					
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	authors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	-						
Taxa, Species, Age:	Vertebrate; I	Fish; Oreochromis niloticus; Juvenile					
Health Outcome:	Mechanistic	-Oxidative stress (including redox biology)	-Immune/He	matological			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3974179						
Domain	ain Metric Rating Comments						
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-changes in HCT, GSH, and MDA.			
	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: 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 or other factors that could influence the outcome assessment. 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 methods were described under the "Data Analysis" section of the paper.			
	Metric 22:	Reporting of Data	Medium	Control and exposure responses for oxidative stress were reported in Table 1. The HCT results for the control and the exposure were only reported in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1 and in the text.			
Additional Comments:	This portion cal/immunit	of the evaluation was on the effect of I y were chosen.	OBP on MD	A, GSH, and HCT. The mechanistic outcomes for oxidative stress and hematologi-			
Overall Quali	ty Deterr	nination	High				

Study Citation:	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species						
Duration	Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. Overall Duration: $0 - 4$ days (0.96b): Exposure Duration: $0 - 4$ days (0.96b).						
Exposure Route.	Aquatic (fres	hwater): Water: Not determined by study a	uthors (i.e.)	(0-201) chemical of interest in exposure water, but unable to determine exact untake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Vertebrate: Fish: Oreochromis niloticus: Invenile					
Health Outcome:	Hepatic/Live	Hepatic/Liver					
Chemical:	Dibutyl phtha	alate (DBP)					
HERO ID:	3974179						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied.			
	Metric 3:	Test Substance Purity	Low	The purity/grade was not reported.			
Domain 2: Test Design	N		TT' 1				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control.			
	Metric 5:	Negative Control Response	High	come of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.			
Domain 3. Exposure Ch	proctarization						
Domain 5. Exposure Ch	Metric 7	Experimental System/Test Media	Low	The preparation of the test media and test concentration was not reported. Exposure was			
	Mettie 7.	Preparation	Low	conducted in 60L glass aquaria.			
	Metric 8:	Consistency of Exposure	High	All exposures were for either 24 or 96h in 60L tanks at 23C with 10 fish per tank.			
		Administration	_				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be up to 96h with sampling done at 24h and 96h. This was appropriate for the outcome of interest.			
	Metric 11:	Number of Exposure Groups/	N/A	The goal of the study was not to have a dose response, but to observe the histological			
		Spacing of Exposure Levels		effect of one sublethal concentration of DBP on the fish.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentration was below the water solubility limit.			
Domain 4. Test Organise	n						
Zomani i. Test Organisi	Metric 13:	Test Organism Characteristics	High	The tilapia were from the Cukurova University Freshwater Fish Production Station in			
	N 14		TT' 1	Adana, Turkey and were juveniles.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The tilapia were acclimated for 15d prior to the start of the study.			
	Metric 15:	Number of Organisms and	Medium	There were 10 fish per test chamber and 2 tanks per treatment per exposure duration.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The tilapia were kept in 60L tanks with 10 fish per tank at 23C with a 16L:8D photone-			
		and Transform and the sound stores	8	riod. They were fed commercial trout food at 2% their body weight.			
		Conti	nued on nex	t page			
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HERO ID: 3974179 Table: 4 of 5

continued from previous page							
Study Citation:	Erkmen, B., Nile tilapia (Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96n); Exposure Duration	on: 0 - 4 days				
Exposure Koute,	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:	X 7 / 1 / T						
Taxa, Species, Age:	Vertebrate; F	rish; Oreochromis niloticus; Juvenile					
Health Outcome:	Hepatic/Live						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3974179						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in liver histology.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Liver and gill tissue was fixed at the end of the study and examined via microscope for histological changes.			
Domain 6: Confounding / Variable Control							
	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	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were described under the "Data Analysis" section of the paper.		
	Metric 22:	Reporting of Data	High	Control and exposure responses are reported in Figure 2 and were adequate for the out- come of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	: This portion of the evaluation was on the effect of DBP on the liver. Histopathology was performed, so the liver/hepatic outcome was chosen.					

Overall Quality Determination High

Study Citation: Duration: Exposure Route, Madia Dath:	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. 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>Oreochromis niloticus</i> ; Juvenile Development/Growth Dibutyl phthalate (DBP) 3974179					
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. The DBP was reported to be from Merck, but it was not reported to be analytically veri- fied		
	Metric 3:	Test Substance Purity	Low	The purity/grade was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in the text.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test media and test concentration was not reported. Exposure was conducted in 60L glass aquaria.		
	Metric 8:	Consistency of Exposure	High	All exposures were for either 24 or 96h in 60L tanks at 23C with 10 fish per tank.		
	Metric 9:	Administration Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be up to 96h with sampling done at 24h and 96h. This was appropriate for the outcome of interest.		
	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 the oxidative stress effect of one sublethal concentration of DBP on the fish.		
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentration was below the water solubility limit.		
Domain 4: Test Organis	n					
	Metric 13:	Test Organism Characteristics	High	The tilapia were from the Cukurova University Freshwater Fish Production Station in Adana, Turkey and were juveniles.		
	Metric 14:	Acclimatization and Pretreatment	High	The tilapia were acclimated for 15d prior to the start of the study.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 10 fish per test chamber and 2 tanks per treatment per exposure duration.		
Domain 5: Outcome Ass	Sessment Metric 16:	Adequacy of Test Conditions	High	The tilapia were kept in 60L tanks with 10 fish per tank at 23C with a 16L:8D photope- riod. They were fed commercial trout food at 2% their body weight.		
	Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 3974179 Table: 5 of 5

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media Path:	Erkmen, B., Nile tilapia (Overall Dura Aquatic (free	Erkmen, B., Benli, K., A.C., Agus, H. H., Yildirim, Z., Mert, R., Erkoc, F. (2017). Impact of sublethal di-n-butyl phthalate on the aquaculture fish species Nile tilapia (Oreochromis niloticus): Histopathology and oxidative stress assessment. Aquaculture Research 48(2):675-685. 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: Oreochromis niloticus: Iuvenile				
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3974179					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth.		
	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	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 Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were described under the "Data Analysis" section of the paper.		
	Metric 22:	Reporting of Data	Low	Control and exposure responses were reported in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion	of the evaluation was on the effect of DBF	o developmen	t/growth.		
Overall Quali	ty Detern	nination	High			

Study Citation: Duration: Exposure Route,	Khalil, , S. F in juvenile N Overall Dura Aquatic (free	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80. 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:	Vertebrate; F Renal/Kidne Dibutyl phth 3350208	Vertebrate; Fish; <i>Oreochromis niloticus</i> ; Juvenile Renal/Kidney Dibutyl phthalate (DBP) 3350208						
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 99% by the manufacturer.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent control group, but did not indi- cate if that control group represented DMSO or not. So, no solvent control or negative control was indicated, just a control. Also, authors did not report the amount of DMSO used for treatment groups.				
	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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes.				
Domain 2: Exposure Ch	aractorization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided few details on exposure media preparation. The acclimation period detailed glass aquaria but no details on vessel media type for sub-lethal tests were provided.				
	Metric 8:	Consistency of Exposure	Medium	No mention of irregularities in exposure administration.				
	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 suitable for the study type.				
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and spacing of exposure levels were suitable for a dose				
		Spacing of Exposure Levels		response although only concentrations were tested.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations (5.9 and 3.9 mg/L) were at or below the water solubility limit (11.2 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. Mean size was reported as 10.6 grams.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for two weeks.				
	Continued on next page							

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HERO ID: 3350208 Table: 1 of 5

continued from previous page							
Study Citation:	Khalil, , S. R	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage					
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 au	thors (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; Oreochromis niloticus; Juvenile					
Health Outcome:	Renal/Kidne	V					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3350208						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Exposures were conducted with two replicates of 10 fish each for treatment and control exposures.			
Domain 5: Outcome Assessment							
	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: 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			
	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 with ANOVA analysis and a Duncans post-hoc.			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in Figure 5.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This form is	for the histopathological assessment of kidn	ey tissue.				

Overall Quality Determination

Medium

Study Citation:	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage					
	in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.					
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; <i>Oreochromis niloticus</i> ; Juvenile					
Health Outcome:	Respiratory					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	3350208					
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 99% by the manufacturer.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent control group, but did not indi-		
				cate if that control group represented DMSO or not. So, no solvent control or negative		
				control was indicated, just a control. Also, authors did not report the amount of DMSO		
				used for treatment groups.		
	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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	Low	The study provided few details on exposure media preparation. The acclimation period		
		Preparation		detailed glass aquaria but no details on vessel media type for sub-lethal tests were pro-		
	Matria 8.	Consistency of Exposure	Madium	Vided.		
	Methic 8.	A dministration	Ivieululli	No mention of megularities in exposure 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 suitable for the study type.		
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and spacing of exposure levels were suitable for a dose		
		Spacing of Exposure Levels		response although only concentrations were tested.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations (5.9 and 3.9 mg/L) were at or below the water solubility limit (11.2 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. Mean size was reported as 10.6 grams.		
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two weeks.		
	Metric 15.	Conditions Number of Organisms and	Medium	Exposures were conducted with two replicates of 10 fish each for treatment and control		
		Replicates per Group	mourum	exposures.		
		Card	nued on most	<u>.</u>		
Continued on next page						

on

HERO ID: 3350208 Table: 2 of 5

-						
Study Citation:	Khalil, , S. R in juvenile N	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis piloticus). Japanese Journal of Veterinary Research 64(1):67-80				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	1: 0 - 4 days (0-9	6h)		
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; Oreochromis niloticus; Juvenile				
Health Outcome:	Respiratory					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3350208					
Domain	Metric Rating Comments					
Domain 5: Outcome Asse	essment					
	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: 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 Presentat	ion and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described with ANOVA analysis and a Duncans post-hoc.		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in Figure 5.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
	This form is for the histopathological assessment of gill tissue.					

Study Citation:	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage						
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (free	In juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80. 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; I	Vertebrate; Fish; Oreochromis niloticus; Juvenile					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	aling/function-O	xidative stress (including redox biology)-Cardiovascular-Endocrine toxicity-Liver			
Chemical: HERO ID:	toxicology Dibutyl phth 3350208	alate (DBP)					
Domain	2220200	Metric	Rating	Comments			
Domain 1: Test Substan	ce		U				
	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 99% by the manufacturer.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent control group, but did not indi- cate if that control group represented DMSO or not. So, no solvent control or negative control was indicated, just a control. Also, authors did not report the amount of DMSO used for treatment groups.			
	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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
Domain 3: Exposure Ch	naracterization						
ľ	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided few details on exposure media preparation. The acclimation period detailed glass aquaria but no details on vessel media type for sub-lethal tests were pro- vided.			
	Metric 8:	Consistency of Exposure	Medium	No mention of irregularities in exposure administration.			
	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 suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and spacing of exposure levels were suitable for a dose			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations (5.9 and 3.9 mg/L) were at or below the water solubility limit (11.2 mg/L).			
Domain 4: Test Organism							
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. Mean size was reported as 10.6 grams.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two weeks.			
	Metric 15:	Conditions Number of Organisms and	Medium	Exposures were conducted with two replicates of 10 fish each for treatment and control			
		Replicates per Group		exposures.			
Continued on next page							

Environmental Hazard Evaluation

		contin	ued from previ	ous page		
Study Citation:	Khalil, , S. R in juvenile N	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-9	96h)		
Exposure Route,	Aquatic (fres	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; Oreochromis niloticus; Juvenile				
Health Outcome:	Mechanistic-	-Biomarkers (exposure and effect)-Cell signa	aling/function-O	xidative stress (including redox biology)-Cardiovascular-Endocrine toxicity-Live		
	toxicology					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3350208					
Domain		Metric	Rating	Comments		
Domain 5: Outcome Ass	aamant					
Domain 5. Outcome Asse	Matric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adaquate		
	Metric 17:	Adequacy of Test Conditions	Low	The outcome assessment mathedology reported the intended outcome of interest		
	Metric 17.	Consistency of Outcome	High	Outcome assessment methodology reported the intended outcome of interest.		
	Wieute 18.	Assessment	Ingn	Outcomes were assessed consistently across study groups.		
Domain 6: Confounding	/ Variable Cor	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.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Presenta	tion and Anal	vsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described with ANOVA analysis and a Duncans post-hoc.		
	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.		

Study Citation:	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage					
	in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.					
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:	Vertekente Fiele One den sie statione Investig					
Taxa, Species, Age:	Vertebrate; Fish; <i>Oreochromis niloticus</i> ; Juvenile					
Chamical	Hepatic/Liver					
HERO ID.	3350208	alate (DBF)				
Demein	5550200	Matuia	Datina	Commente		
Domain Domain 1: Test Substan	<u></u>	Metric	Rating	Comments		
Domain 1. Test Substan	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 nurity reported as 90% by the manufacturer		
	Metrie 5.	Test Substance Fully	Ingn	chemical party reported as 55 % by the manufacturer.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent control group, but did not indi- cate if that control group represented DMSO or not. So, no solvent control or negative control was indicated, just a control. Also, authors did not report the amount of DMSO used for treatment groups.		
	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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes.		
Domain 3: Exposure Ch	aracterization		_			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided few details on exposure media preparation. The acclimation period detailed glass aquaria but no details on vessel media type for sub-lethal tests were provided.		
	Metric 8:	Consistency of Exposure	Medium	No mention of irregularities in exposure administration.		
	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 suitable for the study type.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable for a dose response although only concentrations were tested		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations (5.9 and 3.9 mg/L) were at or below the water solubility limit (11.2 mg/L).		
Domain 4: Tast Organia	m					
Domani 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. Mean size was reported as 10.6 grams.		
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two weeks.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Exposures were conducted with two replicates of 10 fish each for treatment and control exposures.		
	Continued on next page					

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HERO ID: 3350208 Table: 4 of 5

Study Citation: Khalil, S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80. Duration: 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) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oreochromis niloticus; Juvenile Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Metric 18: Consistency of Outcome High Obmain 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. Domain 7: Data Presentation and Analysis Low The study did not provide enough information to the study to suggest differences among groups.	continued from previous page						
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; Oreochromis niloticus; Juvenile Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment Low Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Methoology Metric 18: Consistency of Outcome High Outcome sasessment methoology reported the intended outcome of interest. Domain 6: Confounding / Variable Control Assessment Design and Procedures Metric 20: Domain 7: Data Presentation and Analysis Metric 10:	Study Citation:	Khalil, , S. H in juvenile N	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.				
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) Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oreochromis niloticus; Juvenile Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment 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. Domain 6: Confounding / Variable Control Assessment Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 7: Data Presentation and Analysis Media Test Low The rew as no information in the study to suggest differences among groups.	Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)		
Media, Path: Taxa, Species, Age: Vertebrate; Fish; Oreochromis niloticus; Juvenile Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment Low Metric 17: Outcome Assessment Methodology Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome High The outcome assessment methodology reported the intended outcome of interest. Metric 18: Consistency of Outcome Metric 19: Confounding Variables in Test Design and Procedures Medium Metric 20: Outcomes Unrelated to Exposure Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups.	Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study at	uthors (i.e., chem	nical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age: Vertebrate; Fish; Oreochromis niloticus; Juvenile Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment 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 The outcome assessment methodology are assessed consistently across study groups. Domain 6: Confounding / Variable Control Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 7: Data Presentation and Analysis Medical to Exposure Medium There was no information in the study to suggest differences among groups.	Media, Path:						
Health Outcome: Hepatic/Liver Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment Low Environmental conditions were not sufficiently reported to evaluate if adequate. 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. Domain 6: Confounding / Variable Control Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 7: Data Presentation and Analysis Medium There was no information in the study to suggest differences among groups.	Taxa, Species, Age:	Vertebrate; I	Fish; Oreochromis niloticus; Juvenile				
Chemical: Dibutyl phthalate (DBP) HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment Low Environmental conditions were not sufficiently reported to evaluate if adequate. 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: Confounding / Variable Control Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 6: Confounding / Variable Control Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 7: Data Presentation and Analysis Medium There was no information in the study to suggest differences among groups.	Health Outcome:	Hepatic/Live	er				
HERO ID: 3350208 Domain Metric Rating Comments Domain 5: Outcome Assessment 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 Assessment Outcomes were assessed consistently across study groups. 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. Domain 7: Data Presentation and Analysis Metric 20: Studied by the study did not provide to the test by the study to suggest differences among groups.	Chemical:	Dibutyl phth	alate (DBP)				
Domain Metric Rating Comments Domain 5: Outcome Assessment 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 Assessment Assessment Low The study did not provide enough information to allow a comparison of environmental conditions. 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. 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 Domain 6: Confourding and Analysis Metric 10: Scriptic Metric 10: Metric 10:	HERO ID:	3350208					
Domain 5: Outcome Assessment 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 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. 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 10: Metric 10: Metric 10:	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. Domain 6: Confounding / Variable Control Assessment 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 Presentation and Analysis Metric 10: Statistic 1M the horizon of the study of the	Domain 5: Outcome A	ssessment					
Metric 17: Outcome Assessment Methodology Metric 18: High Consistency of Outcome High High The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Low The study did not provide enough information to allow a comparison of environmental conditions. Domain 7: Data Presentation and Analysis Metric 19: Strict I Metric 10: Metric 10:		Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.		
Metric 18: Consistency of Outcome High Outcomes were assessed consistently across study groups. Domain 6: Confounding / Variable Control Image: 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. Domain 7: Data Presentation and Analysis Image: Confounding Variable Analysis Image: Confounding Variable Analysis		Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study to suggest differences among groups.		Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
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 environmental 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 20: Statistic I Metric I			Assessment				
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. Domain 7: Data Presentation and Analysis Metric 20: Statistic IM the horizontal to Exposure Metric 20:	Domain 6: Confoundin	g / Variable Co	ntrol				
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 Metric 21 Statistical Metric 21		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. Domain 7: Data Presentation and Analysis High how the base of the study of the st			Design and Procedures		conditions.		
Domain 7: Data Presentation and Analysis		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 with ANOVA analysis and a Duncans post-hoc.	Domain 7. Dua Hesen	Metric 21:	Statistical Methods	High	Statistical methods were adequately described with ANOVA analysis and a Duncans post-hoc.		
Metric 22: Reporting of Data High Data for exposure-related findings were presented for each treatment and control group in Figure 4.		Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in Figure 4.		
Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes.		Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		

Additional Comments: This form is for the histopathological assessment of liver tissue.

Overall Quality Determination

Medium

Study Citation:	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage						
	in juvenile N	in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80.					
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:	X 7 / 1 / T						
Taxa, Species, Age:	Vertebrate; F	Mortelity					
Health Outcome:	Mortality	alata (DDD)					
HFRO ID:	3350208	alate (DBP)					
	5550200						
Domain Domain 1: Test Substan		Metric	Rating	Comments			
Domain 1: Test Substan	ce Matria 1:	Test Substance Identity	Uich	The shaming was identified by some and CAS#			
	Metric 1: Metric 2:	Test Substance Source	Low	The test substance identity was not analytically varified by the performing laboratory			
	Metric 2.	Test Substance Source	Low	Chamical musitu was reported as 00% from the membraturer.			
	Metric 5.	Test Substance Fullty	High	Chemical purity was reported as 99% from the manufacturer.			
Domain 2: Test Design							
2 onum 21 100 2 oorgi	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent control group, but did not indi- cate if that control group represented DMSO or not. So, no solvent control or negative control was indicated, just a control. Also, authors did not report the amount of DMSO used for treatment groups.			
	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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
Domain 3: Exposure Ch	aracterization		-				
	Metric /:	Experimental System/Test Media Preparation	Low	The study provided few details on exposure media preparation. The acclimation period detailed glass aquaria but there were no details on vessel media type for the mortality tests.			
	Metric 8:	Consistency of Exposure Administration	Medium	There was no mention of irregularities in exposure administration. Authors did not report if the control group also had DMSO.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured, and water replacement was not detailed for the mortality portion of the study.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable 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	Five of the lower concentrations of DBP were below solubility listed in the scope (11.2), while seven of the treatment concentrations were above solubility.			
Domain 4: Test Organis	m						
Domani 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. Mean weight was reported as 10.6 grams.			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions for two weeks.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	No replicates were used. Guideline, OCSPP 850.1075, recommends two replicates of ten fish each.			
Continued on next page							

HERO ID: 3350208 Table: 5 of 5

continued from previous page							
Study Citation:	Khalil, , S. R., Elhakim, Abd, Y., El-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage						
Duration	in juvenile Nile tilapia (Oreochromis niloticus). Japanese Journal of Veterinary Research 64(1):67-80. Overall Duration: $0 = 4$ days (0.96b): Exposure Duration: $0 = 4$ days (0.96b)						
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; Oreochromis niloticus; Juvenile						
Health Outcome:	Mortality						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	3350208						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Assessment							
	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: 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 environmental 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 Low Statistical methods were adequately described, however, the LC50 method used dia allow for the calculation of error terms surrounding the LC50 value obtained.							
	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 Commanta: An LC50 value was obtained (11.9 mg/L) that is ever the solubility for this commound (11.2 mg/L)							

Additional Comments: An LC50 value was obtained (11.8 mg/L) that is over the solubility for this compound (11.2 mg/L).

Overall Quality Determination

Medium
Study Citation: Duration: Exposure Route, Madia Bath:	Patyna, P. J. Overall Dura Aquatic (fres	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; Fish; <i>Oryzias latipes</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 5489073						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.			
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.			
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was 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 for development are reported for control groups.			
	Metric 6:	Randomized Allocation	Low	Allocation was not random.			
Domain 3: Exposure Ch	naracterization						
r in r	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	Measured concentrations are reported in table 4.2/4.3, but developmental observations were not reported for each concentration.			
	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/ Spacing of Exposure Levels	Low	The incidence of lesions are reported below and above the LC50 for DBP, so it is not able to relate to a dose-response gradient.			
	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 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:	Number of Organisms and Replicates per Group	Low	The authors report the exposures were done in triplicate, however, sample numbers reported in the results do not reflect replicated sample numbers (Table 4.2-4.3) pages 90,91/158.			

Dibutyl Phthalate

HERO ID: 5489073 Table: 1 of 4

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HFRO ID:	Patyna, P. J. (Overall Dura Aquatic (fres Vertebrate; F. Development Dibutyl phtha 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 Dibutyl phthalate (DBP)					
Domain	0.00000	Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment		6				
	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	Low	The authors described morphological lesions from DBP exposure but failed to present data on concentration specific incidences.			
	Metric 18:	Consistency of Outcome Assessment	Low	It is not clear how developmental morphology was scored at each concentration and control for the lesions listed in table 4.5 (page 94/158). Data are not presented.			
Domain 6: Confounding	/ Variable Con	itrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.			
Domain 7: Data Presenta	ation and Analy	ysis					
	Metric 21:	Statistical Methods	Uninformative	The authors report the presence of lesions with no data on quantifying at difference exposure concentrations.			
	Metric 22:	Reporting of Data	Low	There is not enough data on incidence of lesions to relate to a dose-response gradient.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for lesions at specific concentrations to determine.			
Additional Comments:	This evaluation	This evaluation form is relevant to the morphological lesions results given on page 94/158.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 5489073 Table: 2 of 4

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (fres	(1999). Reproductive effects of phthalate ttion: 11 - 21 days; Exposure Duration: 4 shwater); Water; Not determined by study	esters in Japa - 10 days authors (i.e.,	nese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	ish; Oryzias latipes; Embryo		
Health Outcome:	Mortality			
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	5489073			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.
Domain 2. Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	The authors reported performing control and solvent controls.
	Metric 5:	Negative Control Response	High	Control responses are acceptable and presented in Table 4.2 and 4.3 (Pages 90.91/158).
	Metric 6:	Randomized Allocation	Low	Allocation was not random.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Hıgh	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	High	The test compound was verified and concentration gradients are reported as measured concentrations in Tables 4.2 and 4.3 (pages 90.91/158).
	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/ Spacing of Exposure Levels	Medium	The authors report five measured concentrations that span relevance in no effects to complete mortality. No indication of prior range finding were reported.
	Metric 12:	Testing at or Below Solubility Limit	High	All concentrations reported for DBP were under the solubility reported in the final scope (11 mg/l).
Domain 4. Test Organis	m			
Domain 1. Test organis	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:	Number of Organisms and Replicates per Group	Low	The authors report the exposures were done in triplicate, however, sample numbers reported in the results do not reflect replicated sample numbers (Table 4.2-4.3) pages 90,91/158.

Dibutyl Phthalate

			F			
Study Citation: Duration: Exposure Route, Media, Path:	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 Mortality Dibutyl phth 5489073	Fish; <i>Oryzias latipes</i> ; Embryo nalate (DBP)				
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	Outcome assessment methodology reported the intended outcomes (hatch, mortality, development) appropriately for these embryo exposures.		
	Metric 18:	Consistency of Outcome Assessment	High	The assessments were conducted similarly across treatment and control groups.		
Domain 6: Confoundin	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Authors report using USEPA Probit analysis ver 1.4 for LC and EC50 calculations.		
	Metric 22:	Reporting of Data	Low	Authors report LC50 for mortality with no Confidence Intervals and no other compo- nents of the dose-response curve (NOEC, LOEC). Percent 3 day survival was also given (Table 4.3) without any measures of variability.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.		

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 5489073 Table: 3 of 4

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (fre	(1999). Reproductive effects of phthalate ation: 11 - 21 days; Exposure Duration: 4 shwater); Water; Not determined by study	esters in Japa - 10 days authors (i.e.,	nese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; I Renal/Kidne Dibutyl phth 5489073	Fish; <i>Oryzias latipes</i> ; Embryo ey nalate (DBP)		
Domain	5107075	Metric	Rating	Comments
Domain 1: Test Substan	ce		Tutting	
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The authors reported performing control and solvent controls.
	Metric 5:	Negative Control Response	High	Control responses are acceptable and presented in Table 4.2 and 4.3 (Pages 90,91/158).
	Metric 6:	Randomized Allocation	Low	Allocation was not random.
Domain 3: Exposure Ch	aracterization			
1	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	High	The test compound was verified and concentration gradients are reported as measured concentrations in Tables 4.2 and 4.3 (pages 90,91/158).
	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/ Spacing of Exposure Levels	Medium	The authors report five measured concentrations that span relevance in no effects to complete mortality. No indication of prior range finding were reported.
	Metric 12:	Testing at or Below Solubility Limit	High	All concentrations reported for DBP were under the solubility reported in the final scope (11 mg/l).
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	All pretreatment conditions were the same for control and exposed organisms. Embryo media and test water conditions were reported on page 45/158.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors report the exposures were done in triplicate, however, sample numbers reported in the results do not reflect replicated sample numbers (Table 4.2-4.3) pages 90,91/158.

Dibutyl Phthalate

		conti	nued from p	revious page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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 Renal/Kidney Dibutyl phthalate (DBP) 5489073				
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	nigii	lesions) appropriately for these embryo exposures.	
	Metric 18:	Consistency of Outcome Assessment	High	The assessments were conducted similarly across treatment and control groups.	
Domain 6: Confounding	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.	
Domain 7: Data Presen	tation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Authors report using USEPA Probit analysis ver 1.4 for LC and EC50 calculations.	
	Metric 22:	Reporting of Data	Low	Authors report EC50 for urinary bladder lesions (page 50/158) with no Confidence Intervals and no other components of the dose-response curve (NOEC, LOEC).	
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.	
Additional Comments:	This evaluati	on form is relevant to the urinary bladder i	nflation resu	Its results given in Table 4.2.	

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	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:	Age: Vertebrate; Fish; Oryzias latipes; Embryo							
Health Outcome:	Cardiovascul	Cardiovascular Dibutul phthelate (DBB)						
Chemical:	Dibutyl phthalate (DBP)							
HERO ID:	5489073							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. The CAS number was not reported.				
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. The source was reported as from Sigma Aldrich.				
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was 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 for development are reported for control groups.				
	Metric 6:	Randomized Allocation	Low	Allocation was not random.				
Domain 3 [.] Exposure Ch	aracterization							
Domain of Exposure on	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:	Administration Measurement of Test Substance Concentration	Medium	Measured concentrations are reported in table 4.2/4.3, but developmental observations were not reported for each concentration.				
	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/ Spacing of Exposure Levels	Low	The incidence of lesions are reported below and above the LC50 for DBP, so it is not able to relate to a dose-response gradient.				
	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 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	The authors report the exposures were done in triplicate, however, sample numbers reported in the results do not reflect replicated sample numbers (Table 4.2-4.3) pages 90, 91/158.				
Domain 5: Outcome Ass	sessment							

Dibutyl Phthalate

HERO ID: 5489073 Table: 4 of 4

		CO	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Patyna, P. J. Overall Dura Aquatic (fres Vertebrate; F Cardiovascu	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 Cardiovascular				
HERO ID:	Dibutyl phthalate (DBP) 5489073					
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	Low	The authors described morphological lesions from DBP exposure but failed to present data on concentration specific incidences.		
	Metric 18:	Consistency of Outcome Assessment	Low	It is not clear how developmental morphology was scored at each concentration and control for the lesions listed in table 4.5 (page 94/158). Data are not presented.		
Domain 6: Confounding	g / Variable Co	ntrol				
·	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	The results were qualitative.		
	Metric 22:	Reporting of Data	Uninformative	Not sufficient reporting of results (Table 4.5).		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for cardio effects at specific concentrations to determine if there are any unexpected outcomes.		
Additional Comments:	This evaluati	ion form is relevant to the blood flow and eder	ma histo results.			

Overall Quality Determination

Uninformative

Study Citation:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).						
Duration: Exposure Poute	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11	- 21 days	nical of interact in exposure water, but unable to determine exact untake route)			
Media, Path:	Aquatic (iie	sinwater), water, not determined by study a	iutions (n.e., chem	ited of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vertebrate; I	Fish; Oryzias latipes; Adult					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064186						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT ¹ 1				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.			
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory. Chamical purity was reported as 00.2%			
	Wette 5.	Test Substance I unity	Ingn	Chemical purity was reported as 99.5 %.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Study authors used a 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	Matria 7	Experimental System/Test Media	Low	The study manufold only limited details on the measures taken to encountristaly manage			
	Metric 7.	Preparation	LOW	test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on the results.			
		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 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 Organia							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The test organisms were not adequately described, and they were assumed to be from			
	Methe 15.	Test organism characteristics	Low	the same source as the definitive test.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.			
	Matria 15.	Conditions	Madian				
	Metric 15:	Replicates per Group	Medium	i the numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.			
		Replicates 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	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Continued on next page						

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Dibutyl Phthalate

HERO ID: 10064186 Table: 1 of 2

		continu	ued from previo	bus page			
Study Citation:	EAG Labora	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).					
Duration:	Overall Dura	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; F	Vertebrate; Fish; Oryzias latipes; Adult					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064186						
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 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.			
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:	This evaluati	This evaluation is for a range-finder test.					

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	EAG Labora Overall Dura Aquatic (fres	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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; Fish; <i>Oryzias latipes</i> ; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP)						
HERO ID:	10064186						
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	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors used a 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						
	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 or measurements were not reported.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organis	m						
-	Metric 13:	Test Organism Characteristics	Low	The test organisms were not adequately described and assumed to be same source as definitive test			
	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 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	Low	Environmental conditions were not sufficiently reported to evaluate if adequate			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported			
		Assessment		reporteu			

Dibutyl Phthalate

HERO ID: 10064186 Table: 2 of 2

		contir	nued from previo	us page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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> ; Adult						
Health Outcome:	Reproductive	e/ leratogenic					
HERO ID:	10064186						
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 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			
Domain 7: Data Present	ation and Anal	ysis					
	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:	range finder						
Overall Quali	Overall Quality Determination Medium						

Study Citation: Duration:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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:	Vertebrater Fiele Organize India on Adult					
Health Outcome:	Reproductive	-Ish; <i>Oryzias lanpes</i> ; Adult e/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	High	The test substance identity was analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%		
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	Medium	The study reported that organisms were randomly allocated into study groups		
	, .					
Domain 3: Exposure Ch	aracterization		TT: 1			
	Metric /:	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 Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals		
	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		
	Matric 12.	Spacing of Exposure Levels	High	response		
	Wieute 12.	Testing at of below Solubility Linit	Ingn	Exposure concentrations were below the water solubility minit		
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		
	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					
Domain 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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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 1 of 8

continued from previous page						
Study Citation:	EAG Labora	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).				
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; F	Fish; Oryzias latipes; Adult				
Health Outcome:	Reproductiv	e/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	U			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups		
Demain 7. Data Daraan						
Domain 7: Data Presen	Matria 21.	ysis Statistical Matheda	Illah	Cardistical matheds many advanced by described		
	Metric 21:	Statistical Methods	nign II:-h	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:	generational	effects				
Overall Quali	ty Deterr	nination	High			

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HERO ID: 10064186 Table: 2 of 8

Study Citation: Duration: Exposure Route, Media Path:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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 Mechanistic Dibutyl phth 10064186	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Endocrine toxicity-Reproductive/Teratogenic Dibutyl phthalate (DBP) 10064186					
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	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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 organisms were randomly allocated into 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:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals			
	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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response Exposure concentrations were below the water solubility limit			
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	High	The test organisms were acclimatized to test conditions			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group					
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			

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 2 of 8

continued from previous page						
Study Citation: Duration: Exposure Route,	EAG Labora Overall Dura Aquatic (free	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). Overall Duration: > 21 days; Exposure Duration: > 21 days Aquetic (freehwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)				
Media, Path:	1		~ /			
Taxa, Species, Age:	Vertebrate; F	Fish; Oryzias latipes; Adult				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	naling/functi	on-Endocrine toxicity-Reproductive/Teratogenic		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	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	unexpected outcomes were satisfactorily explained		
Additional Comments:	generational	effects				
Overall Quality Determination High						

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Study Citation: Duration: Exposure Route, Media, Path:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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; Oryzias latipes; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064186						
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	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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			
	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 methods for preparation of test media were described in			
	metric /.	Preparation	mgn	adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and			
	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			
	metale II.	Spacing of Exposure Levels	mgn	response			
	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 adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions			
	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 Ass	sessment						
Jomani J. 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			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 3 of 8

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Study Citation: Duration: Exposure Route, Media, Path:	EAG Labora Overall Dura Aquatic (free	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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; Oryzias latipes; Adult				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	TT: 1	1 100		
	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	unexpected outcomes were satisfactorily explained		
Additional Comments:	generational	effects				
Overall Quality Determination			High			

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HERO ID: 10064186 Table: 4 of 8

Study Citation: Duration: Exposure Route, Media Path:	EAG Labora Overall Dura Aquatic (fre	e generation reproduction test (final report). chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate; I	Vertebrate; Fish; Oryzias latipes; Adult						
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	10064186							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#				
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.				
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%				
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 organisms were randomly allocated into study groups				
Domain 3 [.] Exposure Ch	naracterization							
Domain 5. Exposure of	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				
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and				
	Matria 10:	Concentration Exposure Duration and Erequency	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				
	Wieute 11.	Spacing of Exposure Levels	Ingn	response				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit				
Domain 4: Test Organis	sm							
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	The test organisms were acclimatized to test conditions				
	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							
Domain 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				
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups				

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 4 of 8

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	EAG Labora Overall Dura Aquatic (frea Vertebrate: F	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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)				
Health Outcome:	Mortality	,				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	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	unexpected outcomes were satisfactorily explained		
Additional Comments: generational effects				_		
Overall Quality Determination		High				

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Study Citation: Duration: Exposure Route, Media Path:	EAG Labora Overall Dura Aquatic (fre	atories, (2018). Dibutyl phthalate: Medaka ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	a extended one 1 days authors (i.e.,	e generation reproduction test (final report). chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate: I	Fish: Orvzias latines: Adult		
Health Outcome:	Hepatic/Live	er		
Chemical:	Dibutyl phth	nalate (DBP)		
HERO ID:	10064186			
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	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.3%.
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 organisms were randomly allocated into 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
	Wetter 7.	Preparation	Ingn	adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
	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	ingn	response.
	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 adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.
	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			
	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 (pages 37-39).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 5 of 8

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Study Citation: Duration: Exposure Route, Media Path:	EAG Labora Overall Dura Aquatic (free	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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 Hepatic/Live Dibutyl phth 10064186	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Hepatic/Liver Dibutyl phthalate (DBP) 10064186				
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 differences among 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 (Appendix 8).		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	This is for th	e liver histopathology evaluation.				
Overall Quali	ty Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 10064186 Table: 6 of 8

Study Citation: Duration: Exposure Route, Media Path:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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	ish: Orvzias latipes: Adult			
Health Outcome:	Skin & Conr	nective Tissue			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	10064186				
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	High	The test substance identity was analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%	
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	
	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	High	exposures were administered consistently across study groups	
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals	
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable 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	High	Exposure concentrations were 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	High	The test organisms were acclimatized to test conditions	
	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 (page 37)	
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 6 of 8

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path:	EAG Labora Overall Dura Aquatic (free	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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	ish; Oryzias latipes; Adult				
Health Outcome:	Skin & Coni	alata (DRP)				
HERO ID:	10064186					
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 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:	ts: This is for the anal fin papillae evaluation.					
Overall Quality Determination			High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Madia Path:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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	Vertebrate; Fish; Oryzias latipes; Adult					
Health Outcome:	Renal/Kidne	y					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064186						
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	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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	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	High	The experimental system and methods for preparation of test media were described in			
	Weute 7.	Preparation	mgn	adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals			
	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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response Exposure concentrations were below the water solubility limit			
Domain 4: Test Organis	m						
_ sham 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	High	The test organisms were acclimatized to test conditions			
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group					
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 (pages 37-39)			
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups			

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 7 of 8

continued from previous page						
Study Citation: Duration: Exposure Route,	EAG Labora Overall Dura Aquatic (free	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Renal/Kidney Dibutyl phthalate (DBP) 10064186					
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	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 (Appendix 8)		
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained		
Additional Comments:	This is for th	e kidney histopathology evaluation.				
Overall Quality Determination		High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 10064186 Table: 8 of 8

Study Citation: Duration: Exposure Route, Madia Bath:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). 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: Taya Species Age	Vertebrate: F	Vertebrate: Fish; Oryzias latipes; Adult					
Health Outcome:	Endocrine	ish, Oryzius impes, Mun					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	10064186						
Domain		Metric	Rating	Comments			
Domain 1: Test Substa	nce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#			
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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 reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
Domain 3 [.] Exposure C	haracterization						
Domain 5. Exposure e	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			
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals			
	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			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response Exposure concentrations were below the water solubility limit			
Domain 4: Test Organi	sm Matri - 12	Test Organism Characteristics	TT:-L				
	Metric 13:	Acclimatization and Pretreatment	High High	The test organisms were adequately described and were obtained from a reliable source.			
	Wieure 14.	Conditions	mgn	The test organisms were accumulated to test conditions			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group					
Domain 5: Outcome A	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 (pages 37-39)			
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups			

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 10064186 Table: 8 of 8

continued from previous page						
Study Citation:	EAG Labora	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study an	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; Adult				
Health Outcome:	Endocrine					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064186					
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	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 (Appendix 8)		
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained		
Additional Comments:	This is for th	e thyroid gland histopathology evaluation.				
Overall Quality Determination		High				

Study Citation: Duration: Exposure Route, Media. Path:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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; Fish; Oryzias latipes; Adult					
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5489073					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.		
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.		
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.		
	Metric 5:	Negative Control Response	High	Control growth/final weight at 180 days of feeding exposure within Table 6.1 on page 100/158.		
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.		
Domain 2. Expansion Ch	anastanization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.		
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.		
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.		
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	High	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page $46/158$)		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.		
Continued on next page						

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Dibutyl Phthalate

HERO ID: 5489073 Table: 1 of 11

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route,	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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: Taya Species Age:	Vartabrata: F	Sish: Opyrige latings: Adult				
Health Outcome:	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Development/Growth					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5489073					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.		
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology reported the intended outcomes (growth) appropri- ately for these dietary exposures.		
	Metric 18:	Consistency of Outcome Assessment	High	The assessments were conducted similarly across treatment and control groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. Authors did a poor job reporting the output from statistical tests and lack significance results (p values, DF, F scores).		
	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	No unexpected outcomes appear to have been encountered.		
Additional Comments:	This evaluati	ion form is relevant to growth endpoints- to	otal body wei	ght, gonad weight and gonadal somatic index results (Table 6.1) for F1 generation		

Overall Quality Determination

High

Study Citation: Duration: Exposure Route,	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mechanistic Dibutyl phth 5489073	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP) 5489073						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation.				
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.				
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.				
	Metric 5:	Negative Control Response	Hıgh	Control VTG is reported in results section on page 65/158 and figure 6.1 on page 128/158.				
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.				
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.				
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.				
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.				
Domain 4: Test Organisi	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	High	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.				

Domain 5: Outcome Assessment

Dibutyl Phthalate

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. (Overall Dura Aquatic (fres Vertebrate; F Mechanistic- Dibutyl phtha 5489073	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP) 5489073					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.			
	Metric 17:	Outcome Assessment Methodology	Medium	Methods for western blot analysis for vitellogenin were provided and this method pro- vided qualitative data.			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment was conducted similarly across treatment and control groups.			
Domain 6: Confounding	/ Variable Con	trol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.			
Domain 7: Data Presenta	ation and Analy	vsis					
	Metric 21:	Statistical Methods	N/A	VTG was not quantified but rather reported for its presence/absence (Western Blots) in males exposed to dietary concentrations of DBP.			
	Metric 22:	Reporting of Data	Low	Results were not quantitative. Western blot figures were not clear to interpret results but were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability occurred.			
Additional Comments:	No VTG was	detected for DBP treatment group in F0 n	nales.				

Overall Quality Determination

Low

Study Citation: Duration: Exposure Route,	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mechanistic Dibutyl phth 5489073	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP) 5489073					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation.			
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.			
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.			
	Metric 5:	Negative Control Response	Low	Control VTG was not reported for F1 generation. The representative western blot figures provided were not clear to interpret control data.			
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.			
Domain 3 [,] Exposure Ch	aracterization						
Domain of Exposure on	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.			
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.			
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.			
Domain 4: Test Organisi	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	High	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.			

Domain 5: Outcome Assessment

Dibutyl Phthalate

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. Overall Dura Aquatic (fres Vertebrate; F Mechanistic Dibutyl phth 5489073	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP) 5489073				
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.		
	Metric 17:	Outcome Assessment Methodology	Medium	Methods for western blot analysis for vitellogenin were provided and this method pro- vided qualitative data.		
	Metric 18:	Consistency of Outcome Assessment	High	The assessment was conducted similarly across treatment and control groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	N/A	VTG was not quantified but rather reported for its presence/absence (Western Blots) in males exposed to dietary concentrations of DBP.		
	Metric 22:	Reporting of Data	Low	Results were not quantitative. Representative western blot analysis figures provided in the dissertation were not clear to interpret results that were described in the text.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.		

Additional Comments: VTG was detected for DBP treatment group in males at the highest treatment concentration (776 mg/kg/d). (Page 66/158)

Overall Quality Determination

Low

Study Citation: Duration:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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, Modia Dath:								
Taxa, Species, Age:	Vertebrate; Fish; Oryzias latipes; Adult							
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5489073	5489073						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation.				
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.				
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.				
Demain 2. Test D								
Domain 2: Test Design	Metric 4.	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier				
	Metric 5:	Negative Control Response	Low	Control VTG was not reported for F2 generation. The representative western blot figures				
				provided were not clear to interpret control data.				
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The diet study with multiple generations of exposure was described in detail on page				
		Preparation		46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.				
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.				
	Matric 0.	Administration	High	The test compound in the dist was varified with GC MS and was reported as final con				
	Metric 9.	Concentration	Ingn	centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.				
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.				
	Metric 11:	Number of Exposure Groups/	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that				
		Spacing of Exposure Levels		these concentrations are all mostly likely "several orders of magnitude" above environ- mentally relevant concentrations.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.				
Domain 4: Test Organis	m		TT' 1					
	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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).				
	Metric 15:	Number of Organisms and	Low	The treatment and control groups were conducted in duplicate.				
		Replicates per Group						

Domain 5: Outcome Assessment

Dibutyl Phthalate

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. Overall Dura Aquatic (fres Vertebrate; F Mechanistic- Dibutyl phth 5489073	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP) 5489073					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.			
	Metric 17:	Outcome Assessment Methodology	Medium	Methods for western blot analysis for vitellogenin were provided and this method yielded qualitative data. The highest treatment group from previous generations was not present as the F1 were not able to reproduce.			
	Metric 18:	Consistency of Outcome Assessment	High	The assessment was conducted similarly across treatment and control groups.			
Domain 6: Confounding	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	N/A	VTG was not quantified but rather reported for its presence/absence (Western Blots) in males exposed to dietary concentrations of DBP.			
	Metric 22:	Reporting of Data	Low	Results were not quantitative. Figures in the dissertation were not clear to interpret results but were described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability occurred.			
Additional Comments:	Vitellogenin DBP. The hig generation.	Vitellogenin was not quantified but rather reported for its presence/absence (Western Blots) in males and females exposed to dietary concentrations of DBP. The highest treatment group from previous generations was not present as the F1 were not able to reproduce. Control VTG was not reported for F2 generation.					

Overall Quality Determination

Low
PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (free	(1999). Reproductive effects of phthalate ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	nese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age: Health Outcome:	Vertebrate; F Renal/Kidne	(ish; <i>Oryzias latipes</i> ; Adult					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	5489073						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.			
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.			
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.			
	Metric 5:	Negative Control Response	High	Control kidney histology is presented in figure 6.4.4 on page 136/158.			
	Metric 6:	Randomized Allocation	LOW	Allocation was not reported as random.			
Domain 3: Exposure Ch	aracterization						
I IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.			
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.			
	Metric 9:	Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.			
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.			
Domain 4: Test Organisi	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 Conditions	High	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.			
		r ··· r· - ··r					

Domain 5: Outcome Assessment

Dibutyl Phthalate

	continued from previous page				
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Renal/Kidney Dibutyl phthalate (DBP) 5489073				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.	
	Metric 17:	Outcome Assessment Methodology	High	Abnormal kidney pathology was noted for the highest dietary treatment and is presented on page 67/158 of the results and shown in figures 6.4.5 and 6.4.4. (pages 139,140/158)	
	Metric 18:	Consistency of Outcome Assessment	High	The assessment was conducted similarly across treatment and control groups.	
Domain 6: Confoundin	g / Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.	
Domain 7: Data Presen	tation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	The results are presented as pathology/histology operations among treatment concentra- tions. Traditional statistics were not applied.	
	Metric 22:	Reporting of Data	Low	Results were not quantitative. Abnormal kidney pathology was noted for the highest dietary treatment and is presented on page 67/158 of the results and shown in figures 6.4.5 and 6.4.4. (pages 139,140/158)	
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes appear to have been encountered.	

qualitative and the figures in the pdf were not clear to verify findings.

Overall Quality Determination Low

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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 Reproductive Dibutyl phth 5489073	rish; <i>Oryzias latipes</i> ; Adult e/Teratogenic alate (DBP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.	
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.	
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.	
Domain 2: Test Design					
Domain 2. Test Design	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.	
	Metric 5:	Negative Control Response	High	Control Egg production and GSI values at 180 days of feeding exposure are presented within Table 6.1, 6.2 on page 100,101/158. Page 140/158 has a figure with control egg output.	
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.	
Domain 2. Evenance Ch	anastanization				
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.	
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.	
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.	
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Two exposure concentrations were used. The previous highest feeding group (776 mg/kg/d) did not produce progeny for the F2 generation. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.	
Domain 4: Test Organics	m				
Domain 4: Test Organis	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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi-	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.	
		Conti	nued on nex	xt page	

Dibutyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP) 5480073					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.		
	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	The assessment was conducted similarly across treatment and control groups.		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. Authors did a poor job reporting the output from statistical tests and lack significance results (p values, DF, F scores).		
	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	No unexpected outcomes appear to have been encountered.		
Additional Comments:	There were 1 mg/kg/d) did	no significant differences in reproduction for I not produce progeny for the F2 generation	or the F2 ger 1.	neration between control, 12, and 65 mg/kg. The previous highest feeding group (776		

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Patyna, P. J. Overall Dura Aquatic (free Vertebrate; F Reproductiv Dibutyl phth	(1999). Reproductive effects of phthalate ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study Fish; <i>Oryzias latipes</i> ; Adult e/Teratogenic lalate (DBP)	esters in Japa 1 days authors (i.e.,	nese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)
HERO ID:	5489073			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Hıgh	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.
	Metric 5:	Negative Control Response	High	Control Egg production and GSI values at 180 days of feeding exposure are presented within Table 6.1, 6.2 on page 100,101/158. Page 140/158 has a figure with control egg output.
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.
Domain 2: Euroquina Ch	anatanization			
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.
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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.

Dibutyl Phthalate

... continued from previous page **Study Citation:** Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. **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; Oryzias latipes; Adult **Health Outcome:** Reproductive/Teratogenic Chemical: Dibutyl phthalate (DBP) **HERO ID:** 5489073 Domain Metric Comments Rating Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High Housing conditions for the flow through system with this diet study were appropriate for this bioassay. Metric 17: Outcome Assessment Methodology High Outcome assessment methodology reported the intended outcomes (GSI, Reproductive output) appropriately for these dietary exposures. figure 6.7 on page 141/158 reports eggs per female with notations for significance from control (for the F1 generation). Metric 18: Consistency of Outcome High The assessment was conducted similarly across treatment and control groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test High There was no indication that reported differences were from environmental conditions or Design and Procedures other confounding variables. Metric 20: Outcomes Unrelated to Exposure Medium No information to suggest differences among treatment and control groups attributed to animal health or attrition. Domain 7: Data Presentation and Analysis

				reporting the output from statistical tests and lack significance results (p values, DF, F scores).
	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	No unexpected outcomes appear to have been encountered.
Additional Comments:	This evaluation Table 6.2.	on form is relevant to reproductive output e	ndpoints - t	otal egg production and egg production per female for F1 generation; Results given in

Overall Quality Determination

Metric 21:

Statistical Methods

High

Low

Statistical analysis was performed but not described adequately. Authors did a poor job

Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (free	(1999). Reproductive effects of phthalate ation: > 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study	esters in Japa 1 days authors (i.e.,	nese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Reproductive Dibutyl phth 5489073	Fish; <i>Oryzias latipes</i> ; Adult e/Teratogenic alate (DBP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.
	Metric 5:	Negative Control Response	High	Control Egg production and GSI values at 180 days of feeding exposure are presented within Table 6.1, 6.2 on page 100,101/158. Page 140/158 has a figure with control egg output.
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.
	Metric 9:	Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.
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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.

Dibutyl Phthalate

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Perroductive/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5489073					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.		
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology reported the intended outcomes (Reproductive output- Total egg production and Egg production per female; Table 6.2) appropriately for these dietary exposures. figure 6.6 on page 140/158 reports eggs per female with notations for significance from control.		
	Metric 18:	Consistency of Outcome Assessment	High	The assessment was conducted similarly across treatment and control groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. Authors did a poor job reporting the output from statistical tests and lack significance results (p values, DF, F scores).		
	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	No unexpected outcomes appear to have been encountered.		
Additional Comments:	This evaluation form is relevant to reproductive output endpoints - total egg production and egg production per female for F0 generation; Results given in Table 6.2					

Overall Quality Determination

High

Study Citation: Duration:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation: 137. Overall Duration: > 21 days; Exposure Duration: > 21 days								
Exposure Route, Media. Path:	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; Fish; Oryzias latipes; Adult Development/Growth								
Health Outcome:	Developmen	ut/Growth							
HERO ID:	5489073	5489073							
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce		0						
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. CAS number was not reported.					
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.					
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	The authors reported performing a control diet with ethanol as a carrier.					
	Metric 5:	Negative Control Response	High	Control growth/final weight at 180 days of feeding exposure within Table 6.1 on page 100/158.					
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.					
Domain 3: Exposure Ch	aracterization								
	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.					
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.					
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.					
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.					
		· · ·							
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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).					
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate.					

Domain 5: Outcome Assessment

Dibutyl Phthalate

	continued from previous page					
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Patyna, P. J. Overall Dura Aquatic (frea Vertebrate; F Developmen Dibutyl phth	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Development/Growth Dibutyl phthalate (DBP)				
HERO ID:	5489073					
Domain	M + 1 1 (Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.		
	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	The assessments were conducted similarly across treatment and control groups.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. Authors did a poor job reporting the output from statistical tests and lack significance results (p values, DF, F scores).		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 6.1).		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes appear to have been encountered.		

Additional Comments: No differences in weight within the F2 generation were observed from the control to the 12 and 65 mg/kg/d treatment groups.

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Patyna, P. J. Overall Dura Aquatic (free Vertebrate; F	(1999). Reproductive effects of phthalate e ation: > 21 days; Exposure Duration: > 21 shwater); Water; Not determined by study a Fish; <i>Oryzias latipes</i> ; Adult	sters in Japa days uthors (i.e.,	unese medaka (Oryzias latipes). Doctoral Dissertation:137. chemical of interest in exposure water, but unable to determine exact uptake route)
Health Outcome:	Developmen	t/Growth		
Chemical: HERO ID:	Dibutyl phth 5489073	alate (DBP)		
Domain	0.00000	Metric	Rating	Comments
Domain 1: Test Substand	ce	metric	Rung	connens
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation.
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. Source was reported as from Sigma Aldrich.
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was reported.
Domain 2: Test Design	Maduia A.	Negative Controls	II: -h	
	Metric 4:	Negative Control Response	High High	I ne authors reported performing a control diet with ethanol as a carrier.
	metric 5.	Regulive Control Response	mgn	100/158.
	Metric 6:	Randomized Allocation	Low	Allocation was not reported as random.
Domain 3: Exposure Ch	oractorization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The diet study with multiple generations of exposure was described in detail on page 46/158. The experimental systems are reported and water quality parameters (temp, Dissolved oxygen, pH) were observed in this flow-through system.
	Metric 8:	Consistency of Exposure	High	Exposures were the same across treatments and control groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test compound in the diet was verified with GC-MS and was reported as final con- centrations in the feed as well as the delivery dose for mg/kg/day as 12.2, 65, and 776 mg/kg/d DBP.
	Metric 10:	Exposure Duration and Frequency	High	The dietary treatments were administered after 14 days post hatch and till 180 DPH.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Three exposure concentrations were used. The authors acknowledge on page 68/158 that these concentrations are all mostly likely "several orders of magnitude" above environmentally relevant concentrations.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Dietary study, solubility not applicable.
Domain 4: Test Organisi	n Matri - 12	Tost Ouronism Characteristics	II: -1-	
	Metric 13:	lest 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	The fish were acclimated for two weeks prior to the dietary study beginning. Test condi- tions were the same before and after the start of the study. (page 46/158).
	Metric 15:	Number of Organisms and Replicates per Group	Low	The treatment and control groups were conducted in duplicate and 10 animals per treat- ment were used.

Domain 5: Outcome Assessment

Dibutyl Phthalate

		contii	ued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Patyna, P. J. Overall Dura Aquatic (fres	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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 Dibutyl phth 5489073	ish; <i>Oryzias latipes</i> ; Adult t/Growth alate (DBP)					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions for the flow through system with this diet study were appropriate for this bioassay.			
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology reported the intended outcomes (growth) appropri- ately for these dietary exposures.			
	Metric 18:	Consistency of Outcome Assessment	High	The assessments were conducted similarly across treatment and control groups.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences among treatment and control groups attributed to animal health or attrition.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Authors did a poor job reporting the output from statistical tests and lack significance results (p values, DF, F scores).			
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group. Mean and standard deviation are given for total body weight, gonad weight and gonadal somatic index in Table 6.1			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes appear to have been encountered.			
Additional Comments:	This evaluati	on form is relevant to growth endpoints- to	tal body wei	ght, gonad weight and gonadal somatic index results (Table 6.1) for F0 generation.			

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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; Fish; Oryzias latipes; Adult					
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5489073					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	Nomenclature was reported in section 2.1. The structure was identified in a figure within the dissertation. The CAS number was not reported.		
	Metric 2:	Test Substance Source	High	GCMS was reported on the compound in section 2.1 and the lot number was provided. The source was reported as from Sigma Aldrich.		
	Metric 3:	Test Substance Purity	High	A purity of 99% via GCMS was 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 for development are reported for control groups.		
	Metric 6:	Randomized Allocation	Low	Allocation was not random.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	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 text compound to the containers.		
	Metric 8.	Consistency of Exposure	High	Exposures were the same across treatments and control groups		
	Metale 0.	Administration	mgn	Exposures were the same across treatments and control groups.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	Measured concentrations are reported in table 4.2/4.3, but developmental observations were not reported for each concentration.		
	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/ Spacing of Exposure Levels	Low	The incidence of lesions are reported below and above the LC50 for DBP, so it is not able to relate to a dose-response gradient.		
	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	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	The authors report the exposures were done in triplicate, however, sample numbers reported in the results do not reflect replicated sample numbers (Table 4.2-4.3) pages 90,91/158.		
Domain 5: Outcome Ass	sessment					

PUBLIC RELEASE DRAFT May 2025

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 5489073 Table: 11 of 11

		co	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Patyna, P. J. Overall Dura Aquatic (fre Vertebrate; I Developmer Dibutyl phth 5489073	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka (Oryzias latipes). Doctoral Dissertation:137. 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> ; Adult Development/Growth Dibutyl phthalate (DBP)				
Domain	5105075	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	Low	The authors described morphological lesions from DBP exposure but failed to present data on concentration specific incidences.		
	Metric 18:	Consistency of Outcome Assessment	Low	It is not clear how developmental morphology was scored at each concentration and control for the lesions listed in table 4.5 (page 94/158). Data are not presented.		
Domain 6: Confounding	g / Variable Co	ntrol				
·	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no indication that reported differences were from environmental conditions or other confounding variables.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among treatment and control groups attributed to animal health or attrition.		
Domain 7: Data Present	tation and Anal	lysis				
	Metric 21:	Statistical Methods	Uninformative	The authors report the presence of lesions with no data on quantifying at different expo- sure concentrations.		
	Metric 22:	Reporting of Data	Low	There is not enough data on incidence of lesions to relate to a dose-response gradient.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for lesions at specific concentrations to determine unex- pected outcomes.		
Additional Comments:	This evaluat	ion form is relevant to the morphological lesi	ons results given on page	94/158.		
Overall Quali	ty Deterr	nination	Uninformative			

Study Citation:	Chen, X., Xi	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:	Varitali meter T	Vortebrates Eichs America melasticmas Chell ECED. Lamos				
Taxa, Species, Age: Health Outcome:	Mechanistic	Vertebrate; FISH; <i>Oryztas metastigma</i> ; CngH-EGFF; Larvae Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)	i bilidilig/ legula	ion of receptor activity-Endocrine toxicity-Reproductive/relatogenic		
HERO ID:	2298079					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	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	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	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					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Low	Preparation of test substances and dilution into test medium was not well described		
		Preparation	2011			
	Metric 8:	Consistency of Exposure	High	Exposures appear to have been administered consistently.		
	Matria 0.	Administration	Low			
	Metric 9:	Concentration	LOW	Concentrations are reported as nominal.		
	Metric 10:	Exposure Duration and Frequency	Low	Exposures were 24-hr for embryos, which is shorter than the typical 72-96 hrs utilized in other transgenic 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 DBP) and slightly exceeded solubility (DIDP, DINP, DEHP). Methanol was utilized to increase solubility.		
Domain 4: Test Organisi	m Matric 12:	Test Organism Characteristics	Madium	The source for the transcenie modelse use sited as Chan at al 2007 and Chang and Chan		
	Metric 15.	rest Organisin Characteristics	wiedium	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:	Conditions Number of Organisms and	Medium	Each concentration was tested in triplicate with eight embryos per replicate		
		Replicates per Group		······································		

Dibutyl Phthalate

		contin	ued from previo	us page			
Study Citation:	Chen, X., X phthalates ar	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 obthalates 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	: 0 - 4 days (0-96	h)			
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; Fish; Oryzias melastigma; ChgH-EGFP; Larvae						
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
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 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 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	ysis					
	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. Response for BBP treatment was shown in Figure 4 and co-treatment response for DBP, DEHP, DIDP, and DINP are 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 ap	pplies to BBP, DBP, DEHP, DIDP, and DINP.					
Overall Qualit	ty Deterr	nination	Medium				

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,	Overall Dura Aquatic (fres	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:	Vertebrate; F	ish; Pimephales promelas; Juvenile					
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature reported without CASRN			
	Metric 2:	Test Substance Source	Low	Reported as provided by manufacturer from commercially available batches. Manufac- ture name and batch number not provided. No analytical data reported.			
	Metric 3:	Test Substance Purity	High	At least 95% purity			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative control reported			
	Metric 5: Negative Control Response High Control response accep		Control response acceptable				
	Metric 6:	Randomized Allocation	Low	Allocation method not reported.			
Domain 3: Exposure Ch	aracterization						
	Metric /:	Preparation	Medium	Experimental system well described. However, headspace or measures to prevent volatilization not reported.			
	Metric 8:	Consistency of Exposure	High	Exposure administration consistent across groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Sample extracts were analyzed by gas chromatography at start and end of test.			
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure appropriate for test.			
	Metric 11:	Number of Exposure Groups/	High	Exposure levels appropriate. Range finding test was performed.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Test performed at or below water solubility			
		÷ •					
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Low	Source not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for test 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		vessei.			
Domain 5: Outcome Ass	sessment						
2 ontain 5. Outcome Als	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions appropriate for test.			
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.			
Continued on next page							

Environmental Hazard Evaluation

HERO ID: 1321996 Table: 1 of 2

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):1569-15	574.	
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duratio	n: 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	ish; Pimephales promelas; Juvenile			
Health Outcome:	Mortality				
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	1321996				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	Outcome assessment consistent across groups.	
		Assessment			
Domain 6: Confounding	y / Variable Cor	ntrol			
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.	
		Design and Procedures	6	6 1	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences between groups.	
Domain 7: Data Present	ation and Anal	ysis			
	Metric 21:	Statistical Methods	High	Statistical methods performed and described.	
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints reported.	
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.	
Additional Comments:	None				
Additional Comments.	THORE				
Overall Quality Determination		High			

Study Citation:	Adams, W. J	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. I	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.					
Duration: Exposure Route	A quatic (fre	ation: 0 - 4 days (0-901); Exposure Duran shwater): Water: Not determined by study	on: 0 - 4 days	(0-901) chemical of interest in exposure water, but upple to determine exact uptake route)			
Media Path.	Aquatic (ife	silvater), water, Not determined by study	autions (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa Snecies Age	Vertebrate: F	Fish: Pimenhales promelas: Iuvenile					
Health Outcome	Mortality	isii, 1 inteprates prometas, suvenite					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	metre	Ruting	Comments			
2 chiani 1. Test Substan	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.			
	Metric 2:	Test Substance Source	Low	The source was provided by a manufacturer from commercially available batches. The			
				manufacture name and batch number were not provided. No analytical data was re- ported.			
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.			
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.			
Demain 2. Engenne Ch							
Domain 3: Exposure Ch	Matria 7	Europin antal System /Test Madia	Madium				
	Meuric 7.	Preparation	Medium	with 15 L of test solution. However, headspace or measures to prevent volatilization were not reported.			
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.			
		Administration					
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and 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 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					
	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						
c c	Metric 13:	Test Organism Characteristics	Low	The source was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation 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		vessei.			

Domain 5: Outcome Assessment

Metric 16: Adequacy of Test Conditions

High Environmental conditions were appropriate for the test.

Environmental Hazard Evaluation

HERO ID: 1321996 Table: 2 of 2

	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. 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 (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; Pimephales promelas; Juvenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
	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 Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	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 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 Quality Determination		High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Bencic, D. (C., Flick, R. W., Bell, M. E., Henderson,	W. M., Hua	ng, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.			
	H., Biales, A	I., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential					
	application of	pplication of omics data in risk evaluations under TSCA (internal use only).					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	(0-96h)			
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						
Tava Species Age:	Vertebrate: F	Fish: Pimenhales promelas: Larvae					
Hoalth Outcomo:	Mortality						
Chamical	Dibutul abth	valata (DDD)					
HERO ID:	11581/33						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.			
	Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no infor- mation was given on analytical verification.			
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.			
	Metric 5:	Negative Control Response	High	There were no mortalities in the control test groups.			
	Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trave in the incubator			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure on	Metric 7:	Experimental System/Test Media	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing			
	Medie 7.	Preparation	mgn	design using O-rings was used due to the low water solubility of the test substance. The			
	M		TT' 1	exposure system was adequately described on page 7.			
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Measurements of the test substance water concentrations were carried out using an LC-			
		Concentration		MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe mortality effects.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups was adequate for the experimental design (11 treatment			
		Spacing of Exposure Levels	-	groups), and spacing was appropriate as a NOEC was determined at the end of the study (i.e., lowest concentration was low enough).			
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical- saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.			
		Cont	tinued on ney	at page			
	Continued on next page						

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Environmental Hazard Evaluation

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route,	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae						
Health Outcome:	Mortality Dibutyl phth	Mortality Dibutul abthelete (DDD)					
HERO ID:	11581733						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors did not specifically mention acclimating test organisms prior to the exposure start but culture conditions for all larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water +/- phthalate).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the approved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change			
	Metric 17:	Outcome Assessment Methodology	High	throughout the experiment. Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.			
Domain 6: Confounding	a / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.			
Domain 7. Data Present	ation and Anal	veic					
Domain 7. Data i resell	Metric 21:	Statistical Methods	High	LC50 calculation is described in detail in the supplental document. Also, NOEC deriva- tion was briefly described on page 13 of the report, and Table 2 reported the raw data and percent mortality.			
	Metric 22:	Reporting of Data	High	Table 2 (2B) showed the mortality data across all treatments for all 3 replicates.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not given with the mortality data.			
Additional Comments:	The primary assessment v	y goal of this study was to investigate the with DBP.	potential app	lication of omics data in risk evaluation. This specific evaluation is for the mortality			

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Environmental Hazard Evaluation

HERO ID: 11581733 Table: 1 of 4

		continued from previous page				
Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., H H., Biales, A. D. (2024). A multiomics stud	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential				
	application of omics data in risk evaluations	under TSCA (internal use only).				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	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; Larv	ae				
Health Outcome:	Mortality					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	11581733					
Domain	Metric	Rating	Comments			
Overall Qual	ity Determination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bencic, D. C H., Biales, A application o Overall Dura Aquatic (fres Vertebrate; F Mechanistic- Dibutyl phth 11581733	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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> ; Larvae Mechanistic-Cell signaling/function Dibutyl phthalate (DBP) 11581733				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The chemical name and CASRN were reported. The test substance was purchased from MilliporeSigma (Burlington, MA), but no infor- mation was given on analytical varifaction		
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.		
Domain 2: Test Design						
	Metric 4: Metric 5:	Negative Controls Negative Control Response	High Medium	A control and a control plus O-ring group were tested. The effect measured is gene expression (transcriptomic analysis). There is no gene expression profile established for control/unexposed organisms in this developmental stage for this species, particularly gene expression data from the entire fish. Also, given the nature of the collection of fertilized eggs and an outbred population, gene expression even among unexposed fish is expected to vary. From a sequencing perspective, there were no unexpected results.		
	Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trays in the incubator.		
Domain 3: Exposure Ch	aracterization					
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. The RNA analysis was described on pages 8-10.		
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC- MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe transcriptomic changes.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups (11 treatment groups) and spacing was adequate to		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	obtain the transcriptomic changes and derive a transcriptomic point of departure. Due to the low solubility of the test substance, a passive dosing design using a chemical- saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.		

Domain 4: Test Organism

Environmental Hazard Evaluation

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Study Citation: Duration: Exposure Route, Media, Path:	 Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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; <i>Pimephales promelas</i> ; Larvae						
Health Outcome:	Mechanistic	Mechanistic-Cell signaling/function					
Chemical: HERO ID:	Dibutyl phth 11581733	alate (DBP)					
Domain		Metric	Rating	Comments			
	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors didn't specifically mention acclimating test organisms prior to the exposure start but culture conditions for the larvae were similar to exposure conditions (25 degree C incubator, source of water).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the exposure. For the transcriptomics analysis, there were 4 larvae per replicate. Each individual well contained one larva.			
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the ap- proved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure were used for RNA analysis. The sample processing, RNA isolation, RNA sequencing, and tPOD calculation were described in detail on pages 8-10.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.			
Domain & Confoundin	a / Variabla Co	ntrol					
Bomain o. Comoundin	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	tPOD calculation was described on pages 10 and 11 and seemed appropriate to assess results.			

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Environmental Hazard Evaluation

		continued from	previous page		
Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only).				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Du	ration: 0 - 4 day	s (0-96h)		
Exposure Route,	Aquatic (freshwater); Water; Not determined by st	udy 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:	Dibutyl phthalate (DBP)				
HERO ID:	11581733				
Domain	Metric	Rating	Comments		
	Metric 23: Explanation of Unexpected Outcom	nes High	Measures of variability were given with the tPOD data.		
Additional Comments:	: The primary goal of this study was to investigate the potential application of omics data in risk evaluation. This data evaluation is for the calculation of the transcriptomics-based point of departure following exposure DBP.				
Overall Qualit	ty Determination	High			

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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>; Larvae Behavioral Dibutyl phthalate (DBP) 11581733 				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.	
	Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no infor- mation was given on analytical verification.	
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.	
Domain 2: Test Design	Metric 4.	Negative Controls	High	A control and a control plus O-ring group were tested	
	Metric 5:	Negative Control Response	Low	The goal of the behavior analysis was to calculate a behavior-based point of departure. While the authors reported excluding any inactivity across treatment groups from analy- sis, the authors did not specifically report if the control group behaved as expected.	
	Metric 6: Randomized Allocation Medium Larvae were randomly distributed to chemical exposure beakers and the beaker randomly arranged on trays in the incubator. In addition, for the behavior analy control for positional effects, fish from each of the eight exposure conditions we loaded in order into each of the 6 wells of the first row, with the remaining two placed in the first two positions of the second row; this pattern was subsequent peated across replicate plates.		Larvae were randomly distributed to chemical exposure beakers and the beakers were randomly arranged on trays in the incubator. In addition, for the behavior analysis to control for positional effects, fish from each of the eight exposure conditions were loaded in order into each of the 6 wells of the first row, with the remaining two being placed in the first two positions of the second row; this pattern was subsequently re- peated across replicate plates.		
Domain 2: Exposure Ch	araatarization				
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. After the 24 h exposure live larvae from each exposure vessel were transferred into small plastic weight boats for the behavioral analysis.	
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.	
	Metric 9:	Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC- MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours followed by a behavioral assessment that lasted 28 minutes. This was an adequate exposure time to capture dose-response effects and an adequate behavior assay duration to capture changes in movement patterns.	
Continued on next page					

Environmental Hazard Evaluation

HERO ID: 11581733 Table: 3 of 4

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Study Citation: Duration:	Bencic, D. C H., Biales, A application c Overall Dura	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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:	Variah matar T						
Taxa, Species, Age: Health Outcome:	Rehavioral	rish; <i>Pimephales promelas</i> ; Larvae					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	11581733	()					
Domain		Metric	Rating	Comments			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups was adequate (11 treatment groups). Specific for the behavior assay, authors collected movement data the control, control + O-ring, and the 6 highest treatment groups without significant mortality. The authors did not show movement data across treatment groups; instead they used the data to calculate the behavior point of departure.			
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical- saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.			
Domain 4: Test Organi	sm						
	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors did not specifically mention acclimating test organisms prior to the exposure start but culture conditions for all larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water). Moreover, for the behavior assay, fish were acclimated for 10 min in the dark.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the chemical exposure. For the behavioral analyses, 3 larvae were used per replicate.			
Domain 5: Outcome A	ssessment						
Zoman 5. Outcome A	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the ap- proved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure vessel were transferred into well plates for the behavioral analysis. The behavior analysis was described in detail on page 8 and 13.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.			
Domain 6: Confoundin	ng / Variable Con	ntrol					
		Cont	tinued on nex	xt page			

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 11581733 Table: 3 of 4

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Study Citation: Duration:	Bencic, D. C H., Biales, A application o Overall Dura	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only).				
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; Larvae				
Health Outcome:	Behavioral					
UEDO ID.	Dibutyl phth	alate (DBP)				
HERO ID:	11581755					
Domain		Metric	Rating	Comments		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. For the behav- ior assay, fish across treatment groups were handled in the same manner (e.g., transfer from beakers to well-plates, acclimation period in well-plates). While authors did not specifically mention acclimating test organisms prior to the chemical exposure start, cul- ture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	The behavioral data was analyzed using the ZebraLab software. The bPOD calculation was described in detail on page 14.		
	Metric 22:	Reporting of Data	Medium	bPOD outcomes were described in the text of the results section and Table 6 gives a bPOD value for DBP. However, behavior data (movement patterns) across treatment groups were not included in the report.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not given with the behavioral data.		
Additional Comments:	The primary behavior poi	goal of this study was to investigate the p nt of departure as part of the behavior anal	potential app ysis followin	lication of omics data in risk evaluation. This evaluation is for the calculation of the g exposure to DBP.		

Overall Quality Determination High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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> ; Larvae Mechanistic-Cell signaling/function				
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	11581/33				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	Metric 1:	Test Substance Identity	High	The chamical name and CASPN ware reported	
	Metric 2:	Test Substance Source	Low	The test substance was purchased from MillinoreSigma (Burlington MA) but no infor-	
	metric 2.		Low	mation was given on analytical verification.	
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.	
	Weule 5.	Regarive Control Response	mgn	control/unexposed organisms in this developmental stage for this species, particularly a metabolomics profile from the entire fish. That said, to best evaluate 'normality' of the control response, the metabolite profiles of the fathead minoow larvae with and without o-rings (i.e. control vs. vehicle control) were compared and no discernable differences in their profiles were observed leading to the conclusion of 'no unexpected observed measurements in controls'. Also, given the nature of the collection of fertilized eggs and an outbred population, a metabolomic profile even among unexposed fish is expected to vary. From a spectra analysis perspective, there were no unexpected results.	
	Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trays in the incubator.	
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. The metabolomics analysis procedure was further described on page 11.	
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.	
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC- MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe metabolomic results.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups seemed adequate (11 treatment groups).	
Continued on next page					

Environmental Hazard Evaluation

HERO ID: 11581733 Table: 4 of 4

		cont	inued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path:	Bencic, D. C H., Biales, A application o Overall Dura Aquatic (free	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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; I	Vertebrate; Fish; Pimephales promelas; Larvae					
Health Outcome:	Mechanistic	-Cell signaling/function					
HERO ID:	11581733						
Domain		Metric	Rating	Comments			
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical- saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.			
Domain 4: Test Organi	sm						
	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors didn't specifically mention acclimating test organisms prior to the exposure start but culture conditions for the larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water).			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the exposure. For the metabolomics analysis there were 3 larvae per replicate. Three larvae from each set of the replicate treatments were transferred to individual wells of a 1.0 mL 96-well deep well plate.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the ap- proved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure were used for metabolomics analysis. The metabolite extraction, metabolite derivatization, GC-qToF/MS analysis, and mPOD calculation were described in detail on pages 10-12.			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.			
Domain 6: Confoundir	ng / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.			

Environmental Hazard Evaluation

		conti	nued from p	previous page		
Study Citation: Duration: Exposure Route, Media, Path:	Bencic, D. C H., Biales, A application o Overall Dura Aquatic (free	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only). 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 Dibutyl phth 11581733	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae Mechanistic-Cell signaling/function Dibutyl phthalate (DBP) 11581733				
Domain		Metric	Rating	Comments		
	Metric 20: Outcomes Unrelated to Exposure Medium There was no information given about differences among treatment g that could influence the outcome assessment.					
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Metabolomics data analysis and mPOD calculation was described on pages 11 and 12 and seemed appropriate to assess results.		
	Metric 22:	Reporting of Data	High	Table 5 shows mPOD data and some results are described in the "PODs" section of the results.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability were given with the mPOD data.		
Additional Comments:	The primary goal of this study was to investigate the potential application of omics data in risk evaluation. This data evaluation is for the calculation of the metabolomics-based point of departure following exposure to DBP.					
Overall Quality Determination High						

Study Citation: Duration: Exposure Route, Media. Path:	Bionomics,, I Overall Dura Aquatic (fres	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 route)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Dibutyl phth 1316188	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 1316188				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively by accepted name [di-n-butyl phthalate; DBP]		
	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 con- taining the same dilution water andmaintained under the same conditions as the expo- sure jars, butcontaining no test material, were established."		
	Metric 5:	Negative Control Response	High	No mortalities were observed in the control groups, and the study notes that "The pH values of the test solutions remained comparable to the controls throughout the exposure period (Table "3a). Dissolved oxygen concentrations of the test solutions were comparable to the controls at the testis initiation; however, DO concentrations were reduced with increasing [DBP] concentrations at the end of the exposure."		
	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	Domain 3: Exposure Characterization					
-	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 appro- priate amount of test substance to achieve desired concentration. The 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, and 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 au- thors 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			

Dibutyl Phthalate

HERO ID: 1316188 Table: 1 of 1

	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:	Dibutyl phthalate (DBP)
HERO ID:	1316188

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 ex- posure proups can be compared. The 96hr concentration shows the minimum possible exposure he test organisms were subjected to, but since no intermediate measurements were reported. It is 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 detailed in an appendix – "An aliquot of the concentrate was analyzed by 28-highderbomategraphy with electron capture detection." Metric 10: Exposure Duration and Frequency High Exposure concentrations usand/zeb ty 28-highderbomategraphy with electron capture detection." Metric 11: Number of Exposure Coroups/ Spacing of Exposure Levels Metric 12: High Needifferent ex- used (40, 24, 14, 87 and 5.2 uL/L; nominal concentration). Domain 4: Test Organism Metric 13: Test organism Characteristics Medium Complete details regarding test organism characteristics are lacking. Only the source (cleastruct proto and 4.8 hr acclimatization period inmediately before the test organisms underwent the study). Domain 4: Test Organism Metric 13: Test organism sand Replicates per Group Low Test solution on under semininder appee 2.4 of the study). <th>Domain</th> <th>Metric</th> <th>Rating</th> <th>Comments</th>	Domain	Metric	Rating	Comments
Metric 9: Measurement of Test Substance Concentration Medium Test substance concentrations were only measured at the beginning and end of the study (0-th and 96-br). Analytical methods were apporpriate, and detailed in an appendix capture detection." Metric 10: Exposure Duration and Frequency High Exposure duration is in accordance with the cited methodology [Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1075).] Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels High Exposure concentrations under the limit of vater solubility for DBP were used (40, 24, 14, 8.7 and 5.2 uL/L; nominal concentration). 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, Masschusets") and the mean length and mean weight of the test organ- isms in each fish population lot were reported. Metric 14: Acclimatization and Pretreatment Conditions High Test conditions were adouted in each restand mean weight of the test organisms isms in each fish population lot were reported. Domain 5: Outcome Assessment Metric 16: Number of Organisms and Replicates per Group Low Test conditions were adequate – well detailed on pages 2-4 of the study (16/kr light //khr dark photoperiod; dry food fed ad libitum during the pretreatment preiod; 22/4-1 °c temp: Domain 5: Outcome Assessment Met	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 10: Exposure Duration and Frequency High 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 Five different exposure concentrations under the limit of water solubility for DBP were used (40, 24, 14, 8.7 and 5.2 uL/L; nominal concentration). Domain 4: Test Organism Metric 13: Test organism Characteristics Medium Complete details regarding test organism characteristics are lacking. Only the source Créated minnows were obtained from cultures maintained at EG&G, Bionomics, Wareham, Massachusetts") and the mean length and mean weight of the test organism isms in each fish population lot were reported. Metric 14: Acclimatization and Pretreatment Conditions High All test organisms underwent the same pretreatment process, including an at least 14-day pretreatment period and a 48-br acclimatization period immediately before the tests (described in detail on page 2 of the study). Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low Test conditions were adequate – well detailed on pages 2-4 of the study (16hr light /8hr dark photoperiod; 22+/-1 °c temp; measured water parameters stated).	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 detailed in an appendix – "An aliquot of the concentrate was analyzed by gas-liquidchromatography with electron capture detection."
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: High Testing at or Below Solubility Limit Five different exposure concentrations under the limit of water solubility for DBP were used (40, 24, 14, 8, 7 and 5.2 uLL; nominal concentration). Domain 4: Test Organism 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 mean weight of the test organ- isms in each fish population lot were reported. Metric 14: Acclimatization and Pretreatment Conditions High Metric 15: All test organisms underwent the same pretreatment process, including an at least 14- day pretreatment proid and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study). Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions Low 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).	Metric 10:	Exposure Duration and Frequency	High	Exposure duration is in accordance with the cited methodology [Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).]
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 (l'fathead minnows were obtained from cultures maintained at EG&G, Bionomics, Wareham, Massachusetts") and the mean length and mean weight of the test organism is each fish population lot were reported. Metric 14: Acclimatization and Pretreatment Conditions High All test organisms underwent the same pretreatment process, including an at least 14-day appretreatment period and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study). Metric 15: Number of Organisms and Replicates per Group Low Ten fish were included in each treatment group. It appears two replicates were conducted for each exposure concentration. 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; 32+/-1 °c temp; measured water parameters stated). Low Test conditions were adequate parameters stated). Continued on next page	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Five different exposure concentrations under the limit of water solubility for DBP were used (40, 24, 14, 8.7 and 5.2 uL/L; nominal concentration).
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 an at least 14-day pretreatment period and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study). Metric 15: Number of Organisms and Repericates per Group Low Ten fish were included in each treatment group. It appears two replicates were conducted for each exposure concentration. 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). Low Test conditions were adequate parameters stated).	Metric 12:	Testing at or Below Solubility Limit	High	No exposure concentrations were above the limit of solubility; as such, no solvents were necessary.
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 ach fish population lot were reported. Metric 14: Acclimatization and Pretreatment Conditions High All test organisms underwent the same pretreatment process, including an at least 14-day pretreatment period and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study). Metric 15: Number of Organisms and Low Ten fish were included in each treatment group. It appears two replicates were conducted for each exposure concentration. Domain 5: Outcome Assessment Metric 16: Adequacy of Test Conditions High Metric 16: Explicate 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). Continued on next page Continued on next page	Domain 4: Test Organism			
Metric 14: Acclimatization and Pretreatment Conditions High All test organisms underwent the same pretreatment process, including an at least 14- day pretreatment period and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study). Metric 15: Number of Organisms and Replicates per Group Low Ten fish were included in each treatment group. It appears two replicates were con- ducted for each exposure concentration. 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).	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 15: Number of Organisms and Replicates per Group Low Ten fish were included in each treatment group. It appears two replicates were con- ducted for each exposure concentration. 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 Low Extension Detailed on the ext page	Metric 14:	Acclimatization and Pretreatment Conditions	High	All test organisms underwent the same pretreatment process, including an at least 14- day pretreatment period and a 48-hr acclimatization period immediately before the tests (described in detail on page 2 of the study).
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). Continued on next page Event page	Metric 15:	Number of Organisms and Replicates per Group	Low	Ten fish were included in each treatment group. It appears two replicates were con- ducted for each exposure concentration.
Continued on next page	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).
		С	continued on next page	

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Dibutyl 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:	Dibutyl phthalate (DBP)				
HERO ID:	1316188				

Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Uninformative	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 / LC50 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 organisms could range anywhere between there and the highest reported (0hr) concentration. The authors state "LC50 values were calculated using 0-hour analytical results since a significant loss of phthalate ester from solution occurred during many tests. Estimating LC50 values on 0-hour analytical results allows for more equitable comparisons between tests." Since the actual concentration the test organisms were exposed to is unknown, the reported LC50 is not accurate to a specific concentration, but to a widely variable range of potential concentrations lower than the reported LC50.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was done consistently across study groups."The computer program utilized (Stephan, 1978, personal communication) estimated LC50 values using one of three statistical methods in the following order of preference: moving average angle analysis, probit analysis, binomial probability. The method selected was determined by the characteristics of the data base (i.e. presence or absence of test concentrations causing mortality of 100% of the animals in the test population, test concentrations causing mortality of a partial number of animals in the population, etc.). The computer program scanned the data base, identified the most appropriate statistical method and performed the analysis."			
Domain & Confoundin	a / Variabla Co	ntrol					
Domain 6. Comoundan	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 phtha- late esters IE through IN."			
	Metric 20:	Outcomes Unrelated to Exposure	High	No attrition of test organisms unrelated to exposure was reported.			
Domain /: Data Presen	tation and Anal Metric 21.	Statistical Methods	N/A	Statistical analysis for LC50 calculation was selected and performed by a computer			
	wienie 21.	Statistical Methous		program (in order of preference: moving average angle analysis, probit analysis, or binomial probability).			
	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.			
Continued on next page							

Dibutyl Phthalate

HERO ID: 1316188 Table: 1 of 1

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:	Dibutyl phthalate (DBP)						
HERO ID:	1316188						
Domain	Metric	Rating	Comments				
Additional Comments:	Authors note that substantial loss of test material occurred between 0-hr and 96-hr point, but used the 0-hr concentration to calculate the LC50. Tests						
	organisms were actually exposed to an unknown, lower concentration that the 0-hr reported concentration, so this results in an inflated LC50 (the true LC50						
	would be lower than the reported value).						

Overall Quality Determination

Uninformative
Study Citation: Duration: Exposure Route, Media Path:	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)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Dibutyl phth 5774391	Fish; <i>Pimephales promelas</i> ; Juvenile alate (DBP)				
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	Medium	One test substance (DBoP, di-n-butyl ortho) was commercially purchased and of high purity (>99%). The chemical was also synthesized for tests and purity not analytically verified. Iso and tere forms were also synthesized.		
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					
2 oman of 2nposare on	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. DBP 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.		
	Metric 10.	Concentration Exposure Duration and Frequency	High	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 taken to ensure minimal degradation or loss of test sub- stance during experiments.		
Domain 4: Test Organis	m					
	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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Dibutyl Phthalate

continued from previous 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 (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:	Dibutyl phth	alate (DBP)					
HERO ID:	5774391						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).			
		Replicates per Group					
Domain 5: Outcome As	ssessment						
	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 Coi	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	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.			
	Metric 22:	Reporting of Data	Medium	Minimal results were reported, though LC50 values are provided.			
	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 Commentar	A O6h acesta	toxicity toot for DPD (or the ise tore) remain	rtad I C 50 m	aluan Pahaviar was also assassed DPD was tavia to minnawa			
Auutional Comments:	A 9011 acute	toxicity test for DBP (ortilo, iso, tere) repo	neu LC30 Va	nues. Denavior was also assessed. DDP was loxic to mininows.			

Overall Quality Determination

High

Study Citation:	Mccarthy, J.	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental					
Duration:	Overall Dura	ition: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)			
Exposure Route,	Aquatic (fres	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:	Vantalandar T						
Taxa, Species, Age:	Vertebrate; F	Instality					
Chemical:	Dibutyl phth	alate (DRP)					
HERO ID:	1336024						
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	The chemical purity was reported as 99.5 %.			
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	Low	The biological response of the negative control group was not reported for the prelimi- nary test.			
	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	Daily renewals occurred, but few details were provided.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported but was shorter than recommended.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose			
	14	Spacing of Exposure Levels	TT' 1	response.			
	Metric 12:	Testing at or Below Solubility Limit	Hıgh	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 Conditions	High	Pretreatment conditions were the same for control and exposed organisms, but details were limited.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 30-50 embryos per treatment with two replicates.			
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.			
Continued on next page							

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Environmental Hazard Evaluation

		contin	ued from previ	ous page		
Study Citation: Duration:	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179. 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:	Vertebrate: Fish: Pimephales promelas: Embryo					
Health Outcome:	Mortality	·····, · ·····························				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1336024					
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 Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.		
Domain 7: Data Progan	tation and Anal	weig				
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	Low	There was unexpectedly low survival in the negative control.		
Additional Comments:	This form is DBP. Confid	for fathead minnow morality in the prelimin lence intervals (95%) were reported as well.	ary range findin	g test. Results were reported in the text as an LC50 value for 96h of 2.02mg/L for		

Overall Quality Determination

Medium

Study Citation:	Viscient,, Sm	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
Duration: Exposure Route, Media Path	Overall Dura Aquatic (fres	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	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult Mortality					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	10064185						
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	The source of the test substance was reported and the lot number was identified.			
	Metric 3:	Test Substance Purity	Hıgh	Chemical purity was reported as 99.3%.			
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 3: Exposure Ch	aracterization						
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were minimally described.			
	Metric 8:	Consistency of Exposure	Medium	Few details were provided about the flow-through system.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods but were about half of nominals.			
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was suitable for a preliminary study.			
	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	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4. Test Organisi	m						
2 onium in rost organis.	Metric 13:	Test Organism Characteristics	Low	Adults were tested, which is suitable for a preliminary test but not standard for an acute test.			
	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 replicates were reported. The numbers were low and replicates were not used.			
Domain 5: Outcome Ass	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels were not reported			
Continued on next page							

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Environmental Hazard Evaluation

HERO ID: 10064185 Table: 1 of 1

			nucu nom previ	ous page		
Study Citation:	Viscient,, Sn and OECD 2	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350 and OECD 229 guidelines.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 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:	Vertebrate; Fish; Pimephales promelas; Adult					
Health Outcome:	Mortality					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	10064185					
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	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	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	Low	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:	This form is	for the preliminary exposure, which begins	on P. 146 in App	pendix 6.		
Overall Qualit	ty Detern	nination	Medium			

... continued from previous page

Study Citation: Duration: Exposure Route, Modia, 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: 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) Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 1316189						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Dibutyl phtha 1316189							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	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 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	Authors reported the test substance as "100% active ingredient" (doubtful that it is liter- ally one hundred percent but can be taken to mean very pure as received from manufac- turer).				
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 Cha	aracterization							
Ĩ	Metric 7:	Experimental System/Test Media Preparation	Medium	The flow-through regime was described in detail. Stock solutions were prepared daily & maintained throughout the experiment. Observations of droplets of undissolved test substance and cloudy test solution were reported at high concentrations (Table 16).				
	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, 0h, 96h, and 144h.				
	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 outcome(s) of interest (96-h acute toxicity study for fathead minnows, extended to 144h to observe additional mortality as per study design).				
	Metric 11:	Number of Exposure Groups/	High	Numbers and 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)." Some droplets of undissolved phthalate were seen at high concentrations (Table 16). See Appendix I for details of the solubilizing apparatus used to disperse phthalates in the exposure water.				

Domain 4: Test Organism

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1316189 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Bionomics,,	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows (Pimephales promelas) under flow-through conditions.					
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:	T T . 1 . T						
Taxa, Species, Age:	Vertebrate; F	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality Dibutul abthalata (DBD)						
HFRO ID.							
	1310109						
Domain	14 1 12	Metric	Rating	Comments			
	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.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten fish per group with two replicates.			
Domain 5: Outcome As	sessment	Replicates per Group					
Domain 5. Outcome 745	Metric 16:	Adequacy of Test Conditions	High	Test conditions were described in detail & adequate for maintaining the health of P.			
	14.1.17			promelas.			
	Metric 17:	Outcome Assessment Methodology	High	Fish were observed for mortality every 24 hours.			
	Metric 18:	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 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	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but described in limited detail. (Computer program cited as a personal communication using one of three statistical methods for estimating the LC50, but no details of mathematical operations were given).			
	Metric 22:	Reporting of Data	High	Data for all outcomes was presented.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Definitive L	C 50 values (24, 48, 72, 96, 120 and 144 hc	ours) reported	l for DBP.			
Overall Qualit	v Detern	nination	High				

Study Citation:	Mccarthy, J.	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental				
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (fres	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	Fish: Pimephales promelas: Embryo				
Health Outcome:	Mortality	isii, i uneprimes premetais, 2merge				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1336024					
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 99.5 %		
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 response of the negative control group was reported and reasonable for assessed outcomes although the solvent control had poor survival and hatch		
	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	Medium	Daily renewals but few details were provided		
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported but was shorter than recommended		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose		
	Matria 12.	Spacing of Exposure Levels	Hich	response		
	Metric 12:	Testing at of Below Solubility Limit	Figh	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 Conditions	High	Pretreatment conditions were the same for control and exposed organisms, details were limited		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	30-50 embryos per treatment with two replicates		
Domain 5: Outcome Ass	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	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: 1336024 Table: 1 of 1

		contin	ued from previ	ious page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Mccarthy, J. Toxicology a Overall Dura Aquatic (fres Vertebrate; F Mortality Dibutyl phth 1336024	F., Whitmore, D. K. (1985). Chronic toxicit and Chemistry 4(2):167-179. ation: 11 - 21 days; Exposure Duration: 11 - shwater); Water; Not determined by study at Fish; <i>Pimephales promelas</i> ; Embryo alate (DBP)	y of di-n-butyl a 21 days 1thors (i.e., cher	nd di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmenta
Domain	1550024	Metric	Rating	Comments
2011411	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	Low	Results for this criterion should be related to the solvent control. There was statistically- significant morality in the negative control group that may impact results for reproduc- tive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	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	High	Data for exposure-related findings were presented for each treatment and control group
	Wiether 22.		-	• •

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Viscient,, Sm	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
Duration	and OECD 2	and OECD 229 guidelines. 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 untake route)					
Media, Path:	require (reshwater), water, not determined by study authors (i.e., enomical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vertebrate: F	Vertebrate: Fish: Pimenhales promelas: Adult					
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cell sig	naling/functi	on-Reproductive/Teratogenic			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064185	· · ·					
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	High	The source of the test substance was reported and the lot number was identified			
	Metric 3:	Test Substance Purity	Hıgh	Chemical purity reported as 99.3%			
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 organisms were randomly allocated into 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	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			
	Matria 0.	Administration	Uich	Even over a comparison of the second value of the second			
	Metric 9.	Concentration	nigii	exposure concentrations were measured using appropriate anarytical technologies and methods			
	Metric 10.	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for			
	metile 10.	Exposure Duration and Frequency	mgn	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 at or below the water solubility limit			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab			
	Metric 14.	Acclimatization and Pretreatment	High	source all pretreatment conditions were the same for control and exposed organisms			
	1.10010 1 4 .	Conditions	mgn	an procedurion conditions were the same for conditionand exposed organisms			
	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						
Domain 5. Outcome 715.	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health			
		Cont	inued on nex	at nage			
Continueu on next page							

Environmental Hazard Evaluation

HERO ID: 10064185 Table: 1 of 6

continued from previous page						
Study Citation:	Viscient,, Sn	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350				
	and OECD 2	and OECD 229 guidelines.				
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; F	Sish; Pimephales promelas; Adult				
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Cell sign	aling/functi	on-Reproductive/Teratogenic		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064185					
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 Cor	ntrol				
	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		
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 High						

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HERO ID: 10064185 Table: 2 of 6

Study Citation:	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350 and OECD 229 midelines						
Duration: Exposure Route,	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: Taya Species Age:	Vertebrate: F	Vertebrate: Fish: Pimanhalas promalas: Adult					
Health Outcome:	Endocrine	isii, 1 imepitates prometas, Adun					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064185						
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	High	The source of the test substance was reported and the lot number was identified			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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	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	High	The experimental system and methods for preparation of test madia wars described in			
	Wieure 7.	Preparation	Ingn	adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies and			
	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 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			
Domain 4: Test Organis	m		N				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab 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 A-	accmant						
Domain 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							

Environmental Hazard Evaluation

HERO ID: 10064185 Table: 2 of 6

	continued from previous page						
Study Citation:	Viscient,, Sn	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
	and OECD 2	229 guidelines.					
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	Fish; Pimephales promelas; Adult					
Health Outcome:	Endocrine						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064185						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Demeia (; Cenferratio		- 4 - 1					
Domain 6: Confounding	g / Variable Col	ntroi	II:-h				
	Metric 19:		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	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 form ac	counts for the thyroid histology results repo	orted in the	paper.			
<u> </u>		•	TT+ 1				
Overall Quali	ty Detern	nination	High				

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HERO ID: 10064185 Table: 3 of 6

Study Citation:	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
Duration: Exposure Route, Modia Pathy	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: Fish: Pimenhales promelas: Adult					
Health Outcome:	Mortality	isi, i inceptutes prometus, i tuti				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064185					
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	High	The source of the test substance was reported and the lot number was identified		
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%		
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 organisms were randomly allocated into study groups		
Damain 2. Earna ann Ch						
Domain 5: Exposure Ch	Matria 7.	English and all Saustana /Talat Madia	II: -1-			
	Metric /:	Preparation	High	adequate detail		
	Metric 8:	Consistency of Exposure	High	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	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 spacing of exposure levels were suitable for a dose		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response Exposure concentrations were at or below the water solubility limit		
			2	· · · · ·		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab 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. Outrain A						
Domain 5: Outcome As	Sessment	A deguage of Test Conditions	11:-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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Environmental Hazard Evaluation

continued from previous page						
Study Citation:	Viscient,, Sn	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350				
	and OECD 2	and OECD 229 guidelines.				
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; F	Fish; Pimephales promelas; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	10064185					
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	C			
	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:	None					
Overall Quality Determination High						

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HERO ID: 10064185 Table: 4 of 6

Study Citation:	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
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:	Reproductiv	e/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	10064185					
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	The source of the test substance was reported and the lot number was identified.		
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.3%.		
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 reported and reasonable.		
	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	Hıgh	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 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/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.		
Domain 4: Test Organis	m Matri 12		M. "			
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab 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 A -	aggment					
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: 10064185 Table: 4 of 6

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Study Citation:	Viscient,, Sn	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350				
	and OECD 2	and OECD 229 guidelines.				
Duration:	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; F	Fish; Pimephales promelas; Adult				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	10064185					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain & Confounding	Variable Co	atual				
Domain 6: Confounding	g / Variable Col	Conformatione Maniables in Test	II:-h			
	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		
	Methe 20.	Outcomes Onrelated to Exposure	Ingn	There were no differences among groups.		
Domain 7. Data Present	ation and Anal	vsis				
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		
	infeate 25.	Explanation of Chexpected Catcomes	mgn			
Additional Comments:	None					
Overall Qualit	Overall Quality Determination High					

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HERO ID: 10064185 Table: 5 of 6

Study Citation:	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350 and OECD 229 guidelines.						
Duration: Exposure Route, Madia Batha	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)					
Meula, Paul: Tava Spacias Agas	Vertebrate: I	Vartahrata, Eish, Dimanhalas promalas, Adult					
Health Outcome	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064185	()					
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	High	The source of the test substance was reported and the lot number was identified			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
Domain 2: Test Design	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			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
Damain 2. Error over Ch							
Domain 3: Exposure Ch	Matria 7.	Experimental System/Test Madia	High				
	Metric 7:	Preparation	Figh	adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
		Administration					
	Metric 9:	Measurement of Test Substance 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/	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 at or below the water solubility limit			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab 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 As	assemant						
Domain 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			
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Environmental Hazard Evaluation

HERO ID: 10064185 Table: 5 of 6

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Study Citation:	Viscient,, Sn	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350					
	and OECD 2	229 guidelines.					
Duration:	Overall Dura	ation: > 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; Pimephales promelas; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	10064185						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Demein (; Cenferralia		- 4 - 1					
Domain 6: Confounding	g / Variable Col	ntroi	II: -L				
	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:	None						
Overall Qualit	Overall Quality Determination High						

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HERO ID: 10064185 Table: 6 of 6

Study Citation:	Viscient,, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350						
Duration: Exposure Route, Media Path	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	Vertebrate: Fish: Pimenhales promelas: Adult					
Health Outcome:	Behavioral						
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	10064185						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#			
	Metric 2:	Test Substance Source	High	The source of the test substance was reported and the lot number was identified			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
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 reported and reasonable			
	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	High	The experimental system and methods for preparation of test media were described in			
	Metric 8.	Preparation Consistency of Exposure	High	aucquate detain			
	Wieute 0.	Administration	mgn	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	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 spacing of exposure levels were suitable for a dose			
	Matria 12.	Spacing of Exposure Levels	High	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						
U	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described and were obtained from an unnamed lab 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.			
Demain 5: Outer							
Domain 5: Outcome As	sessment		TT: -h				
	Metric 16:	Adequacy of fest 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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HERO ID: 10064185 Table: 6 of 6

			r	Fig.			
Study Citation:	Viscient,, Sn and OECD 2	Viscient, Smithers (2018). Di-n-butyl phthalate - short-term reproduction assay with fathead minnow (Pimephales promelas) following OPPTS 890.1350 and OECD 229 guidelines.					
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	davs				
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; Fish; Pimephales promelas; Adult						
Health Outcome:	Behavioral						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064185						
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	0				
	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:	Additional Comments: This report accounts for behavioral outcomes reported in the definitive test. This includes respiration rate is included in this as it was reported as a behavioral outcome.						
Overall Qualit	Overall Quality Determination High						

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Study Citation:	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 s ation: > 21 days; Exposure Duration: > 21 shwater); Water, Food/Diet; Dietary	fulvidraco (R I days	Cichardson). Journal of Applied Ichthyology 25(6):771-775.		
Taxa, Species, Age:	Vertebrate; F	Sish; <i>Pseudobagrus fulvidraco</i> ; Adult				
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1335887					
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 grade was reported as analytical.		
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	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	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 adequate for a dose		
		Spacing of Exposure Levels	e	response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure is via diet.		
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms used 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. 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 maintenance of organism health.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.		
		Cont	inued on nex	xt page		

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HERO ID: 1335887 Table: 1 of 3

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Study Citation:	Jee, J. H., K cholinesteras	Koo, J. G., Keum, Y. H., Park, K. H., Cho se activity in bagrid catfish, Pseudobagrus f	i, S. H., Ka ulvidraco (F	ing, J. C. (2009). Effects of dibutyl phthalate and di-ethylhexyl phthalate on acetyl-Richardson). Journal of Applied Ichthyology 25(6):771-775.			
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (free	Aquatic (freshwater); Water, Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult						
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1335887						
Domain		Metric	Rating	Comments			
	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	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	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 Quality Determination			High				

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	ation: > 21 days; Exposure Duration: > 21	l days				
Exposure Route, Modia Dathy	Aquatic (free	Aqualic (neshwater), water, Food/Diet, Dietary					
Taxa Snecies Age	Vertebrate: F	sish: <i>Pseudobagrus fulvidraco</i> : Adult					
Health Outcome:	Mortality	isii, i seudobagras jarvaraeo, i adat					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1335887						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	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	Hıgh	Chemical grade 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						
Domain 5. Exposure 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	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 adequate for a dose			
		Spacing of Exposure Levels	0	response			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure is via diet			
Domain 4: Test Organis	m						
0	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	Initial number was adequate, replicates were not used			
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 Assessment	High	Outcomes were assessed consistently across study groups			

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		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	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Aquatic (free	shwater); Water, Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Pseudobagrus fulvidraco; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
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		
		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	Low	Statistical 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		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
Additional Comments:	None					
Overall Quality Determination		High				

Study Citation:	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 Johthyology 25(6):771-775.						
Duration: Exposure Route,	Overall Dura Aquatic (fres	Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water, Food/Diet; Dietary					
Media, Path: Taxa Spacios Ago:	Vartabrata: F	Sish: Psaudahaarus fulvidraaa: Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1335887						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	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	Chemical grade reported as analytical			
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	Medium	The study reported that organisms were randomly allocated into study group			
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			
	Wette 7.	Preparation	mgn	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 adequate for a dose			
		Spacing of Exposure Levels	8	response			
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure is 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 Replicates per Group	Low	Initial number was adequate, replicates were not used			
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			

Continued on next page ...

HERO ID: 1335887 Table: 3 of 3

continued from previous page						
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	cholinesterase activity in bagrid catfish, Pseudobagrus fulvidraco (Richardson). Journal of Applied Ichthyology 25(6):771-775. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater); Water, Food/Diet; Dietary				
Taxa, Species, Age:	Vertebrate; F	ish; <i>Pseudobagrus fulvidraco</i> ; Adult				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	1335887					
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 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	veis				
Domain 7. Data 11030	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 Quali	ty Detern	nination	High			

Study Citation:	Ohtani, H., I	Ohtani, H., Miura, I., Ichikawa, Y. (2000). Effects of dibutyl phthalate as an environmental endocrine disruptor on gonadal sex differentiation of genetic					
Duration: Exposure Route, Media. Path:	males of the Overall Dura Aquatic (free	Males of the frog Rana rugosa. Environmental Health Perspectives 108(12):1189-1193. Overall Duration: 11 - 21 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:	Vertebrate; A	Vertebrate; Amphibian; Rana rugosa; Larvae					
Health Outcome:	Reproductiv	Reproductive/Teratogenic					
Chemical: HERO ID:	676307	lalate (DBP)					
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	The test substance was identified as being from Sigma.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was 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 reported 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. The exposure containers were "2L enameled containers."			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	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	High	Exposure concentrations were below the water solubility limit.			
Domain 4. Test Organis	m						
Domain 1. Test Organis.	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source (bred in-lab from wild caught organisms).			
	Metric 14:	Acclimatization and Pretreatment	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	Low	Fifty tadpoles per group were used, but no replicates were reported.			
Domain 5: Outcome Ag	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Contir	nued on next pa	ge			

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HERO ID: 676307 Table: 1 of 1

		contin	ued from previ	ous page		
Study Citation:	Ohtani, H., Miura, I., Ichikawa, Y. (2000). Effects of dibutyl phthalate as an environmental endocrine disruptor on gonadal sex differentiation of genetic					
Duration	Overall Dur	ation: 11 - 21 days: Exposure Duration: 0 -	4 days (0-96h)	2):1169-1195.		
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; <i>Rana rugosa</i> ; Larvae					
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Dibutyl phth	nalate (DBP)				
HERO ID:	676307					
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 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	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	lvsis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described (Chi-sq 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					

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						
Duration: Exposure Route, Media Path:	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	Vertebrate: F	ish: Salmo mykiss: Juvenile					
Health Outcome	Mortality	isii, buinto mykiss, suvenite					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1321996						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	се						
	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 provided by a manufacturer from commercially available batches. The manufacture's name and batch number were not provided. No analytical data was reported.			
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4.	Negative Controls	High	A negative control was reported			
	Metric 5:	Negative Control Response	High	The control response was accentable			
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported			
	Wette 0.	Kandoniized / mocation	Low	An anocation method was not reported.			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to pre- vent volatilization were not reported.			
	Metric 8:	Consistency of Exposure	High	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 end of the test.			
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration and frequency of exposure were appropriate for the test			
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed			
	metrie II.	Spacing of Exposure Levels	man	Exposure revers were appropriate. It range maning 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	m						
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were acclimated appropriately.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with ten organisms per test vessel.			
Domain 5: Outcome Ass	resement						
Domain 5. Outcome Ass	Metric 16	A dequacy of Test Conditions	High	Environmental conditions were appropriate for the test			
	Metric 17	Outcome Assessment Methodology	High	The intended outcomes were reported			
	wienie 17.	Gattome Assessment Methodology	riigii	rne mienaea outcomes were reportea.			
		Cont	inued on nex	t page			

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Environmental Hazard Evaluation

HERO ID: 1321996 Table: 1 of 1

continued from previous 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	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	uthors (i.e.,	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vertebrate; F	Fish; Salmo mykiss; Juvenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent across groups.		
		Assessment				
Domain 6: Confounding	, / Variable Co	atrol				
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.		
		Design and Procedures	8			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.		
Domain /: Data Present	ation and Anal		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				

Study Citation:	Tollefsen, K	Tollefsen, K. E., Meys, J. F., Frydenlund, J., Stenersen, J. (2002). Environmental estrogens interact with and modulate the properties of plasma sex steroid-				
Duration: Exposure Route, Media. Path:	binding prot 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:	Vertebrate; F	Fish; Salmo salar; Juvenile				
Health Outcome:	Mechanistic	-Cell signaling/function-Receptor binding/	regulation of	f receptor activity-Endocrine toxicity-Reproductive/Teratogenic		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1332592					
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 DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.		
Domain 2. Test Design						
Domain 21 Test Design	Metric 4:	Negative Controls	High	The study authors reported using a negative control in which the vehicle 2-propanol only was used.		
	Metric 5:	Negative Control Response	High	The negative control response was presented in Fig. 2 and was appropriate for the outcome of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the Atlantic salmon were allocated into study groups.		
Damain 2. Enna ann Ch						
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The test system was described as flow-through, but little other details were provided on the test system. The preparation of the DBP concentrations was not reported. It was not reported whether the exposure vessels were made of glass or plastic.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions prevented evaluation of the consistency of the administration. Test chambers were not reported, nor were test volumes. Environmental conditions were not reported either. Ovrevik, Stenersen, Nilssen, Tollefsen (2002) was cited for methods.		
	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:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be two weeks. This appeared adequate to observe a response.		
	Metric 11:	Number of Exposure Groups/	Medium	There were only three exposure groups, which is lower than is typical, but the spacing		
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	appeared accurate to observe a response. The test concentrations were reported in Fig. 2 and they were below the water solubility.		
	Metrie 12.	Testing at or below Solubility Limit	Ingn	limit. It was reported the vehicle 2-propanol was used as well.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the Atlantic salmon was not reported. They were reported to be juveniles, but length and weight were not reported		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions prior to the study.		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 1332592 Table: 1 of 1

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Study Citation:	Tollefsen, K.	Tollefsen, K. E., Meys, J. F., Frydenlund, J., Stenersen, J. (2002). Environmental estrogens interact with and modulate the properties of plasma sex steroid-			
Duration: Exposure Route,	binding prote Overall Dura Aquatic (fres	binding proteins in juvenile Atlantic salmon (Salmo salar). Marine Environmental Research 54(3-5):697-701. 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: Salmo salar: Juvenile			
Health Outcome:	Mechanistic-	Cell signaling/function-Receptor binding/	regulation o	f receptor activity-Endocrine toxicity-Reproductive/Teratogenic	
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	1332592				
Domain	Metric		Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms per test chamber, and the number of replicates were not re- ported. Six to eight individual fish were used in each exposure group.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and the loading rate of the fish. It was not reported if the organisms were fed during the exposure.	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Øvrevik, Stenersen, Nilssen, and Tollefsen (2001) and Tollefsen (2002) were cited for the outcome assessment methodology.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited. Øvrevik, Stenersen, Nilssen, and Tollefsen (2001) and Tollefsen (2002) were cited for the outcome assessment methodology.	

Domain 6: Confounding	g / Variable Con	ntrol
	Metric 19:	Confounding Var

Domain 6: Confounding / Variable Control						
	Metric 19:	.9: Confounding Variables in Test Design and Procedures		It was not reported if the fish were acclimated, nor were the environmental conditions reported.		
	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	tion and Analy	sis				
	Metric 21:	Statistical Methods	High	Figure 2 reports that significance compared to the control group was analyzed using a one-way ANOVA test followed by a Dunnett's post test.		
	Metric 22:	Reporting of Data	High	Exposure and control responses were reported in Fig 2 along with SEM. Fig 1 reported the displacement of [3H]estradiol from the high affinity estradiol binding site in diluted salmon plasma.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures.		

Additional Comments: This evaluation was on the effect of DBP on sex steroid binding proteins in Atlantic salmon and how this chemical modulates the endocrine system through the interaction with these proteins. Mechanistic outcomes for cell signaling/function, endocrine, receptor/binding, and reproduction were chosen as the outcomes of interest.

Overall Quality Determination

Low

Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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; Amphibian; <i>Xenopus laevis</i> ; Larvae Mortality Dibutyl phthalate (DBP)						
Chemical:							
HERO ID:	10064183						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance							
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#			
	Metric 2:	Test Substance Source	High	The source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >=99%			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors used an appropriate concurrent negative control group for the range- finding test.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for the range-finding test.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups for the range- finding test.			
Domain 3: Exposure Ch	aracterization						
Domain of Exposure of	Metric 7:	Experimental System/Test Media Preparation	Low	The report provided only limited details on the measures taken to appropriately prepare test concentrations for the range-finding test. It is unclear if vessel material was consistent between the range-finding test and the definitive test.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration for the range-finding test.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported for the range-finding test.			
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was suitable for a range-finder			
	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 for a range-finding test.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 1: Test Organise	n						
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	Medium	The test organisms used in the range-finder were not adequately described but assumed to be same source as definitive test			
	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	10 organisms were tested per study group in the range-finder, but replication was not reported.			

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Dibutyl Phthalate

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa Species Age:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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)						
Health Outcome:	Mortality						
Chemical: HERO ID:	Dibutyl phthalate (DBP) 10064183						
Domain		Metric	Rating	Comments			
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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for the range-finder were not reported.			
Domain 6: Confounding / Variable Control							
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions for the range-finder			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups for the range-finder			
Domain 7: Data Presentation and Analysis							
	Metric 21:	Statistical Methods	Low	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 for the range-finder			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	This form is evaluating the range-finding test conducted to determine the concentration of 1-BP that causes lethality and morbidity in Xenopus laevis.						
Overall Quality Determination Medium							

Overall Quality Determination

Medium
Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (20 Overall Dura Aquatic (fres	 18). 21-d amphibian metamorphosis assay (ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study at 	(AMA) of dibuty n: 0 - 4 days (0-9 uthors (i.e., chem	l phthalate with African clawed frog, xenopus laevis. 6h) nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae		
Health Outcome:	Behavioral	•		
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	10064183			
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	The source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as $>=99\%$.
Domain 2: Test Design				
-	Metric 4:	Negative Controls	High	Study authors used an appropriate concurrent negative control group for the range- finding test.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for the range-finding test.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups for the range- finding test.
Domain 3: Exposure Ch	aracterization		Ŧ	
	Metric /:	Experimental System/Test Media Preparation	Low	The report provided only limited details on the measures taken to appropriately prepare test concentrations for the range-finding test. It is unclear if vessel material was consis- tent between the range-finding test and the definitive test.
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration for the range-finding test.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported for the range-finding test.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was suitable for a range-finding test.
	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 for a range-finding test.
	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	Medium	The test organisms used in the range-finder were not adequately described but assumed to be from the same source as the definitive test.
	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	Ten organisms were tested per study group in the range-finder, but replication was not reported.
Domain 5: Outcome Ass	sessment			

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		contin	ued from previo	us page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Battelle, (20 Overall Dura Aquatic (fres Vertebrate; A Behavioral Dibutyl phth	 21-d amphibian metamorphosis assay (<i>A</i> ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study au Amphibian; <i>Xenopus laevis</i>; Larvae alate (DBP) 	AMA) of dibutyl :: 0 - 4 days (0-96 tthors (i.e., chemi	phthalate with African clawed frog, xenopus laevis. ^{5h}) ical of interest in exposure water, but unable to determine exact uptake route)
HERO ID:	10064183			
Domain		Metric	Rating	Comments
	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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for the range-finder were not reported.
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 for the range-finder.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups for the range-finder.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	Low	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 for the range-finder.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This form is	for the behavioral outcome reported for the	preliminary test.	
Overall Qualit	y Detern	nination	Medium	

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Gardner, S.	Г., Wood, A. T., Lester, R., Onkst, P. E., Bur	rnham, N., Peryg	in, D. H., Rayburn, J. (2016). Assessing differences in toxicity and teratogenicity					
	of three phth	alates, Diethyl phthalate, Di-n-propyl phtha	late, and Di-n-bu	ityl phthalate, using Xenopus laevis embryos. Journal of Toxicology and Environ-					
	mental Healt	h, Part A: Current Issues $79(2)$:71-82.	0 4 1 (0 0						
Duration:	Overall Dura	ation: 0 - 4 days (0-96n); Exposure Duration	1: 0 - 4 days (0-9						
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; Amphibian; <i>Xenopus laevis</i> ; Embryo									
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Embryo							
Health Outcome:	Mortality								
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	30/0/43								
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	High	The test substance was synthesized by a source other than the manufacturer					
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was not reported.					
Domain 2. Test Design									
Domain 2: Test Design	Metric 4	Negative Controls	High	Study authors reported using an appropriate consurrant pagetive control group					
	Metric 5:	Negative Control Perponse	Low	The biological response of the pagetive control groups use not reported					
	Metric 6:	Randomized Allocation	Low	Passarchers did not report how organisms ware allocated to study groups					
	Wettie 0.	Kandomized Anocation	LOW	Researchers and not report now organisms were anocated to study groups.					
Domain 3 [,] Exposure Ch	aracterization								
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in					
	metho /:	Preparation	mgii	adequate detail					
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups					
		Administration	e						
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured					
	Matria 10	Concentration	Iliah						
	Metric 10.	Exposure Duration and Frequency	High Lliab	The duration of exposure was suitable for the study type					
	Metric 11:	Specing of Exposure Levels	nigii	/ concentrations with a suitable range					
	Metric 12.	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate					
	incure 12.	Testing at of Delow bolability Linit	Ingn						
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	High	all pretreatment conditions were the same for control and exposed organisms					
		Conditions	e						
	Metric 15:	Number of Organisms and	Medium	Two replicates of twenty embryos was suitable					
		Replicates per Group							
Domain 5: Outcome As	secoment								
Domain 5. Outcome As	Metric 16:	A dequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adaptate of was					
	metric 10.	Aucquacy of test conditions	LUW	monitored but not reported					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest					
		~	8						
		Contin	nued on next pa	ge					

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Environmental Hazard Evaluation

HERO ID: 3070743 Table: 1 of 2

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route,	Gardner, S. 7 of three phth mental Healt Overall Dura Aquatic (free	F., Wood, A. T., Lester, R., Onkst, P. E., Burnalates, Diethyl phthalate, Di-n-propyl phthal h, Part A: Current Issues 79(2):71-82. (tion: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study au	nham, N., Peryg .ate, and Di-n-bu : 0 - 4 days (0-9 .thors (i.e., chen	gin, D. H., Rayburn, J. (2016). Assessing differences in toxicity and teratogenicity utyl phthalate, using Xenopus laevis embryos. Journal of Toxicology and Environ- 96h) nical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age: Health Outcome	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Embryo Mortality				
Chemical: HERO ID:	Dibutyl phth 3070743	alate (DBP)			
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were lim- ited but sufficient	
Domain 6: Confounding	/ Variable Cou	atral			
Domain of Comounding	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The study did not provide enough information to allow a comparison of environmental conditions. However, this unlikely would have had substantial impacts on results.	
	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	High	Statistical methods were adequately described	
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were shown for each treatment and control group in graphs	

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	Gardner, S. of three phth mental Heal Overall Dura Aquatic (fre	T., Wood, A. T., Lester, R., Onkst, P. E., Bu nalates, Diethyl phthalate, Di-n-propyl phtha th, Part A: Current Issues 79(2):71-82. ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study a	rnham, N., Peryg alate, and Di-n-bu n: 0 - 4 days (0-9 uthors (i.e., chen	 cin, D. H., Rayburn, J. (2016). Assessing differences in toxicity and teratogenicity ityl phthalate, using Xenopus laevis embryos. Journal of Toxicology and Environ-6h) hical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Developmen Dibutyl phth 3070743	Amphibian; <i>Xenopus laevis</i> ; Embryo ht/Growth halate (DBP)		
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 was synthesized by a source other than the manufacturer.
	Metric 3:	Test Substance Purity	Low	Purity or 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 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	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 suitable for the study type
	Metric 11:	Number of Exposure Groups/	High	Seven concentrations with a suitable range were used for the test
		Spacing of Exposure Levels	mgn	seven concentrations with a saturble range were used for the test.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.
Domain 4. Test Organis	m			
Domain 4. 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	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions	Medium	Two raplicates of twenty embryos was suitable
	Weute 15.	Replicates per Group	Wiedfulli	Two replicates of twenty enforyes was suitable.
		, and the second s		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. pH was monitored but not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		11CU.
		Conti	nued on next pa	ge

PUBLIC RELEASE DRAFT

Environmental Hazard Evaluation

HERO ID: 3070743 Table: 2 of 2

		contin	ued from previ	ous page		
Study Citation: Duration: Exposure Route, Media, Path:	Gardner, S. ' of three phth mental Heal Overall Dura Aquatic (fre	T., Wood, A. T., Lester, R., Onkst, P. E., Bur nalates, Diethyl phthalate, Di-n-propyl phthal th, Part A: Current Issues 79(2):71-82. ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study au	nham, N., Peryg late, and Di-n-bu 1: 0 - 4 days (0-9 1thors (i.e., chem	in, D. H., Rayburn, J. (2016). Assessing differences in toxicity and teratogenicity tyl phthalate, using Xenopus laevis embryos. Journal of Toxicology and Environ- 6h) tical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vertebrate; Amphibian; Xenopus laevis; Embryo					
Health Outcome:	Developmen	Development/Growth				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	30/0/43					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Co Metric 19:	ntrol Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	conditions. There was no information in the study to suggest differences among groups.		
Domain 7: Data Present	tation and Anal	lysis				
	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 in graphs.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Malformatic	ons, length				
Overall Quali	ty Deterr	nination	Medium			

May 2025

Study Citation: Duration: Exposure Route, Media, Path:	Lee, S. K., C ability and in Overall Dura Aquatic (free	Dwens, G. A., Veeramachaneni, D. N. (200, npairs development of Xenopus laevis frogs attion: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study at	5). Exposure to s. Journal of Tox n: 0 - 4 days (0-9 uthors (i.e., chen	low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv- icology and Environmental Health, Part A: Current Issues 68(10):763-772. 6h) nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; A Behavioral Dibutyl phth	Amphibian; <i>Xenopus laevis</i> ; Embryo alate (DBP)		
HEROID:	6/3293			<u> </u>
Domain 1: Test Substan	<u></u>	Metric	Rating	Comments
Domain 1. Test Substan	Metric 1:	Test Substance Identity	High	The DBP was identified by CAS number
	Metric 2:	Test Substance Source	Low	The DBP was reported to be from Sigma Aldrich and a lot number was provided, but it was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.8%.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a DMSO solvent control as well as a negative control using
	incure it	reguire contons	mgn	FETAX solution.
	Metric 5:	Negative Control Response	Low	The behavior of the control group was not reported.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly assigned to treatment and control groups.
Domain 3: Exposure Ch	aracterization			
Domain 3. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	It was reported that stock solutions were prepared every 48h by diluting DBP into DMSO in amber glass containers. The solutions were then vortexed and refrigerated until use. Final concentrations were made every 24h by adding the stock solution to the FETAX solution in appropriate amounts.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered in 100mm pyrex petri dishes with 20mL of solution and 50 embryos in each test chamber. Tests were administered for 96h until stage 46.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if exposure concentrations were measured at any point in the study.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate for the study and produced a dose response.
	Metric 11:	Number of Exposure Groups/	High	There were six exposure concentrations as well as a negative control and a solvent con- trol. Spacing of exposure concentrations was adequate for a response
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO (0.01%) was used as a solvent in the appropriate amount for this study. A solvent control was used as well.
Domain 4: Test Organis	m			
Domain 1, 1050 Organis.	Metric 13:	Test Organism Characteristics	High	Adult broodstock was reported to be from Nasco in Fort Atkinson, WI. The adults were bred and embryos ranging from stages 8-11 were collected from them.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the embryos were acclimated to test conditions in any way.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 50 embryos per test chamber, and all treatments had a total of 300 embryos. The negative control had 600 embryos. All tests were completed in duplicate.
		Conti	nued on next na	

Environmental Hazard Evaluation

HERO ID: 673293 Table: 1 of 3

		contin	ued from previ	ous page			
Study Citation:	Lee, S. K., Owens, G. A., Veeramachaneni, D. N. (2005). Exposure to low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv- ability and impairs development of Xenopus laevis frogs. Journal of Toxicology and Environmental Health, Part A: Current Issues 68(10):763-772.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-9	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:	Vertebrate; A	Amphibian; Xenopus laevis; Embryo					
Health Outcome:	Behavioral	Behavioral					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	673293						
Domain		Metric	Rating	Comments			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	The embryos were kept at 24C for the duration of the study. They did not need to be fee Adult frogs were fed pellets and were kept at 20-24C in 10mMNaCl. The photoperiod was 12L:12D.			
	Metric 17:	Outcome Assessment Methodology	Low	Behavior assessment methodology was not reported.			
	Metric 18:	Consistency of Outcome	Low	It was not reported how behavior was assessed during the exposure.			
		Assessment					
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if any acclimation occurred.			
	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					
	Metric 21:	Statistical Methods	High	Statistical analysis was discussed in the "Statistical Analyses" section of the article.			
	Metric 22:	Reporting of Data	Medium	Behavior results were briefly described in the results section and a little bit in the discussion section, but no behavior data was shown in tables or figures.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study authors did not report any unexpected outcomes. No variability was given for the behavioral results.			

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., C ability and ir Overall Dura Aquatic (fres Vertebrate; A Developmen Dibutyl phth 673293	Dwens, G. A., Veeramachaneni, D. N. (20 npairs development of Xenopus laevis frog ation: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study Amphibian; <i>Xenopus laevis</i> ; Embryo t/Growth alate (DBP)	05). Exposur gs. Journal of on: 0 - 4 days authors (i.e.,	e to low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv- Toxicology and Environmental Health, Part A: Current Issues 68(10):763-772. (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: Metric 2:	Test Substance Identity Test Substance Source	High Low	The DBP was identified by CAS number. The DBP was reported to be from Sigma Aldrich and a lot number was provided, but it was not reported if it was analytically verified
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.8%.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a DMSO solvent control as well as a negative control using
	Metric 5: Metric 6:	Negative Control Response Randomized Allocation	High Medium	The solvent control response and the negative control response are reported in Table1. Embryos were randomly assigned to treatment and control groups.
Domain 2: Exposure Ch	aractorization			
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	It was reported that stock solutions were prepared every 48h by diluting DBP into DMSO in amber glass containers. The solutions were then vortexed and refrigerated until use. Final concentrations were made every 24h by adding the stock solution to the FETAX solution in appropriate amounts.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered in 100mm pyrex petri dishes with 20mL of solution and 50 embryos in each test chamber. Tests were administered for 96h until stage 46.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if exposure concentrations were measured at any point in the study.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was appropriate for the study and produced a dose response.
	Metric 11:	Number of Exposure Groups/	High	There were 6 exposure concentrations as well as a negative control and a solvent control.
	Metric 12:	Testing at or Below Solubility Limit	High	0.01% DMSO was used as a solvent in the appropriate amount for this study. A solvent control was used as well.
Domain 4: Test Organisi	n			
	Metric 13:	Test Organism Characteristics	High	Adult broodstock was reported to be from Nasco in Fort Atkinson, WI. The adults were bred and embryos ranging from stages 8-11 were collected from them.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the embryos were acclimated to test conditions in any way,
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 50 embryos per test chamber, and all treatments had a total of 300 embryos. The negative control had 600 embryos. All tests were completed in duplicate.

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 673293 Table: 2 of 3

		conti	nued from p	revious page		
Study Citation:	Lee, S. K., C	Dwens, G. A., Veeramachaneni, D. N. (200 ppairs development of Xenopus laevis from	05). Exposur	e to low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv		
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	authors (i.e.	chemical of interest in exposure water, but unable to determine exact uptake route)		
Media. Path:	riquite (iie	sitvater), water, rot determined by study (autil015 (1.e.,			
Taxa. Species. Age:	Vertebrate: A	Amphibian: Xenopus laevis: Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	673293					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	The embryos were kept at 24C for the duration of the study. They did not need to be fed. Adult frogs were fed pellets and were kept at 20-24C in 10mMNaCl. The photoperiod was 12L:12D.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-development/growth of embryos as they developed into tadpoles (stage 46).		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. After the exposure, tadpoles were euthanized and refrigerated. They were then examined under a dissecting scope for abnormalities and stage. Length was determined by photographing each tadpole.		
Domain 6: Confounding	y / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	Low	It was not reported if any acclimation occurred.		
	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 discussed in the "Statistical Analyses" section of the article.		
	Metric 22:	Reporting of Data	High	Data for the negative control, the solvent control, and all exposure levels was reported in Table 1. Figure 1 contained data for the control and the exposure concentrations.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mean SE for body length was reported in Table 1.		
Additional Comments:	This portion chosen as the	of the evaluation was on the effect of DF e outcome according to the Eco outcomes of	3P on the gro	owth and development of X. laevis embryos into tadpoles. Development/growth w the development as well as deformities were recorded as endpoints.		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., C ability and in Overall Dura Aquatic (fres Vertebrate; A Mortality Dibutyl phth 673293	Dwens, G. A., Veeramachaneni, D. N. (20 npairs development of Xenopus laevis fro ation: 0 - 4 days (0-96h); Exposure Duratio shwater); Water; Not determined by study Amphibian; <i>Xenopus laevis</i> ; Embryo alate (DBP)	05). Exposur gs. Journal of on: 0 - 4 days authors (i.e.,	re to low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv- Toxicology and Environmental Health, Part A: Current Issues 68(10):763-772. (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: Metric 2:	Test Substance Identity Test Substance Source	High Low	The DBP was identified by CAS number. The DBP was reported to be from Sigma Aldrich and a lot number was provided, but it
				was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.8%.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a DMSO solvent control as well as a negative control using
		e	6	FETAX solution.
	Metric 5:	Negative Control Response	High	The solvent control response and the negative control response are reported in Table1.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly assigned to treatment and control groups.
Domain 2. Euroques Ch	anastanization			
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	It was reported that stock solutions were prepared every 48h by diluting DBP into DMSO in amber glass containers. The solutions were then vortexed and refrigerated until use. Final concentrations were made every 24h by adding the stock solution to the FETAX solution in appropriate amounts.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered in 100mm pyrex petri dishes with 20mL of solution and 50 embryos in each test chamber. Tests were administered for 96h until stage 46.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if exposure concentrations were measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate for the study and produced a dose response.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 6 exposure concentrations as well as a negative control and a solvent control. Spacing of exposure concentrations was adequate for a response.
	Metric 12:	Testing at or Below Solubility Limit	High	0.01% DMSO was used as a solvent in the appropriate amount for this study. A solvent control was used as well.
Domain 1. Test Organis	m			
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	Adult broodstock was reported to be from Nasco in Fort Atkinson, WI. The adults were bred and embryos ranging from stages 8-11 were collected from them
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the embryos were acclimated to test conditions in any way,
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 50 embryos per test chamber, and all treatments had a total of 300 embryos. The negative control had 600 embryos. All tests were completed in duplicate.

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 673293 Table: 3 of 3

		conti	nued from p	revious page		
Study Citation:	Lee, S. K., Owens, G. A., Veeramachaneni, D. N. (2005). Exposure to low concentrations of di-n-butyl phthalate during embryogenesis reduces surviv- ability and impairs development of Xenopus laevis frogs. Journal of Toxicology and Environmental Health, Part A: Current Issues 68(10):763-772.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	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; A	Amphibian; Xenopus laevis; Embryo				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	673293					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	The embryos were kept at 24C for the duration of the study. They did not need to be fed. Adult frogs were fed pellets and were kept at 20-24C in 10mMNaCl. The photoperiod was 12L:12D.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-mortality. LC50 values were determined, and percent mortality was recorded.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Dead embryos were removed and counted every 24h when solutions were changed.		
Domain 6: Confounding	y / Variable Coi	ntrol	τ			
	Metric 19:	Confounding variables in Test	Low	it was not reported if any acclimation occurred.		
	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				
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical analysis was discussed in the "Statistical Analyses" section of the article		
	Metric 22:	Reporting of Data	High	Data for the negative control, the solvent control, and all exposure levels was reported in Table 1.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion an outcome	of the evaluation was on the effect of DBP for the study.	on mortality	of X. laevis embryos. Mortality was monitored every 24h and was therefore chosen		

Study Citation:	Xu, Y., Gye,	Xu, Y., Gye, M. C. (2018). Developmental toxicity of dibutyl phthalate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-						
Duration: Exposure Route, Media, Path:	534. Overall Dura Aquatic (fres	34. 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; A Mechanistic- Dibutyl phth 4829262	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 4829262						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	e Matria 1	Test Sechster of Identities	τ					
	Metric 1: Metric 2:	Test Substance Source	Low	The test substance was identified by name only. The source was reported to be Sigma Aldrich in Seoul, Korea, but it was not reported if it was analytically verified				
	Metric 3:	Test Substance Purity	High	The 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.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 3-5 and was adequate for the outcomes of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.				
Domain 3: Exposure Ch	oractorization							
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static renewal system with renewals every 24h. DMSO was used to prepare the stock solutions, and serial dilutions were used to prepare the test concentrations with FETAX used as the diluent.				
	Metric 8:	Consistency of Exposure Administration	Medium	Well plates were reported to be use, but it was unclear what size they were. There were 4 plates with 40 embryos for each treatment. Treatments were for 48h.				
	Metric 9:	Measurement of Test Substance Concentration	High	Measured concentrations were reported in parenthesis in ppm in the "materials and methods" section.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which was adequate for the outcomes of interest.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 6 exposure groups and the spacing was appropriate to observe a response.				
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO was used as a vehicle solvent at an appropriate level.				
Domain 4: Test Organise	n							
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	The Xenopus laevis were bred at Hanyang University Aquarium. Embryos were used for this study.				
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were bred at the performing laboratory, and lab conditions were appropriate.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 4 wells that contained 40 embryos for each treatment.				
		Replicates per Group						

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Environmental Hazard Evaluation

HERO ID: 4829262 Table: 1 of 3

		conti	inued from p	previous page			
Study Citation:	Xu, Y., Gye, 534	Xu, Y., Gye, M. C. (2018). Developmental toxicity of dibutyl phthalate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	on: 0 - 4 days	s (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:							
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Embryo					
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Genoto	x (including	DNA repair)-Oxidative stress (including redox biology)			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	4829262						
Domain		Metric	Rating	Comments			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Embryos were kept in well plates with 10 embryos each at 23C. FETAX solution was used as the embryo rearing solution and diluent. The solution was renewed daily for the duration of the test.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest_DNA fragmentation oxidative stress reaction mRNA levels			

	1. 10		*** 1	increase of the internation, oxidative sites reaction, increase of the sites
	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 Con	trol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures	e e	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	tion and Analy	sis		
	Metric 21:	Statistical Methods	High	Statistical methods were described in detail in the "statistical analysis" section.
	Metric 22:	Reporting of Data	High	Data for the control and the exposure responses were reported in Figures 3-5 and were
		1 0	e	appropriate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the
			-	figures.
Additional Comments:	This portion of	of the evaluation was on the effect of DBP	on X. laevi	s embryos. Mechanistic outcomes for biomarkers, genotox, and oxidative stress were
	chosen as the	outcomes of interest.		

Overall Quality Determination

High

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HERO ID: 4829262 Table: 2 of 3

Study Citation:	Xu, Y., Gye, M. C. (2018). Developmental toxicity of dibutyl phthalate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-							
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (fre	ation: 0 - 4 days (0-96h); Exposure Durations of the study s	on: 0 - 4 days authors (i.e.,	(0-96h) chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Embryo						
Health Outcome:	Developmen	nt/Growth						
Chemical:	Dibutyl phth	nalate (DBP)						
HERO ID:	4829262							
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 was reported to be Sigma Aldrich in Seoul, Korea, but it was not reported if it was analytically verified.				
	Metric 3:	Test Substance Purity	High	The 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.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 2 and Figures 1 and 2 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.				
Domain 3: Exposure Ch	aracterization	Europimontal System/Test Madia	High	The sustain successful and an effective state of the sustained by the DMCO successful to				
	Metric 7:	Preparation	nıgıi	prepare the stock solutions, and serial dilutions were used to prepare the test concentra- tions with FETAX used as the diluent.				
	Metric 8:	Consistency of Exposure Administration	Medium	Well plates were reported to be use, but it was unclear what size they were. There were four plates with 40 embryos for each treatment. Treatments were for 48h.				
	Metric 9:	Measurement of Test Substance Concentration	High	Measured concentrations were reported in parenthesis in ppm in the "materials and methods" section.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which was adequate for the outcomes of interest.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were six exposure groups and the spacing was appropriate to observe a response.				
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO was used as a vehicle solvent at an appropriate level.				
Domain 4: Test Organis	m							
C	Metric 13:	Test Organism Characteristics	High	The Xenopus laevis were bred at Hanyang University Aquarium. Embryos were used for this study.				
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were bred at the performing laboratory, and lab conditions were appropriate.				
	Metric 15:	Conditions Number of Organisms and Paplicates per Group	Medium	There were four wells that contained 40 embryos for each treatment.				

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Environmental Hazard Evaluation

HERO ID: 4829262 Table: 2 of 3

		conti	nued from p	revious page			
Study Citation:	Xu, Y., Gye,	M. C. (2018). Developmental toxicity of	dibutyl phtha	alate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-			
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:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Embryo					
Health Outcome:	Developmen	t/Growth					
HERO ID:	4829262						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Embryos were kept in well plates with 10 embryos each at 23C. FETAX solution was used as the embryo rearing solution and diluent. The solution was renewed daily for the duration of the test.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–embryo growth and malformations.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. MZ8: Leica, Heerbrugg, Switzerland was cited for malformations and Nieuwkoop and Faber for staging.			
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.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in detail in the "statistical analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the control and the exposure responses were reported in Table 2 and in Figures 1 and 2.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	This portion outcome of i	of the evaluation was on the effect of DI nterest.MZ8: Leica, Heerbrugg, Switzerlar	BP on X. lac	evis embryo length and malformation. Development and growth was selected as the for malformations, and Nieuwkoop and Faber was cited for developmental staging.			

Overall Quality Determination

High

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HERO ID: 4829262 Table: 3 of 3

Study Citation:	Xu, Y., Gye,	Xu, Y., Gye, M. C. (2018). Developmental toxicity of dibutyl phthalate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (fre	(0-96h) chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Vertebrate; Amphibian; Xenopus laevis; Embryo						
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	nalate (DBP)					
	4829202			2			
Domain Domain 1: Tast Substan	22	Metric	Rating	Comments			
Domain 1: Test Substan	Metric 1	Test Substance Identity	Low	The test substance was identified by name only			
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich in Seoul, Korea, but it was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The 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.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and was adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static renewal system with renewals every 24h. DMSO was used to prepare the stock solutions, and serial dilutions were used to prepare the test concentrations with FETAX used as the diluent.			
	Metric 8:	Consistency of Exposure Administration	Medium	Well plates were reported to be use, but it was unclear what size they were. There were 4 plates with 40 embryos for each treatment. Treatments were for 48h.			
	Metric 9:	Measurement of Test Substance Concentration	High	Measured concentrations were reported in parenthesis in ppm in the "materials and methods" section.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which was adequate for the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 6 exposure groups and the spacing was appropriate to observe a response.			
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO was used as a vehicle solvent at an appropriate level.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The Xenopus laevis were bred at Hanyang University Aquarium. Embryos were used for this study.			
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were bred at the performing laboratory, and lab conditions were appropriate.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 4 wells that contained 40 embryos for each treatment.			

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Environmental Hazard Evaluation

HERO ID: 4829262 Table: 3 of 3

		conti	nued from p	revious page			
Study Citation:	Xu, Y., Gye,	Xu, Y., Gye, M. C. (2018). Developmental toxicity of dibutyl phthalate and citrate ester plasticizers in Xenopus laevis embryos. Chemosphere 204:523-					
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:	Vertebrate; A	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Embryo					
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	4829262						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Embryos were kept in well plates with 10 embryos each at 23C. FETAX solution was used as the embryo rearing solution and diluent. The solution was renewed daily for the duration of the test.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality in the form of LC50 values.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Embryos were assessed every 24h and any dead embryos were removed.			
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.			
	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 methods were described in detail in the "statistical analysis" section.			
	Metric 22:	Reporting of Data	High	Data for the control and the exposure responses were reported in Table 1.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. 95% confidence intervals were not provided.			
Additional Comments:	This portion interest.	of the evaluation was on the effect of DB	P on X. laev	is embryos. LC50 values were reported, so mortality was selected as the outcome of			

Overall Quality Determination

High

Study Citation:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis.							
Exposure Route, Media. Path:	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; A	Vertebrate; Amphibian; Xenopus laevis; Larvae						
Health Outcome:	Mortality	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	10064183							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Chemical was identified by name, CAS#, and structure.				
	Metric 2:	Test Substance Source	High	The source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.				
	Metric 3:	Test Substance Purity	High	Chemical purity 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				
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated to tanks, and tanks were randomly 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 suitable and described in adequate detail. Exposure water-contact components included glass, stainless steel, and Teflon.				
	Metric 8:	Consistency of Exposure	High	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	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				
	Metric 12:	Spacing of Exposure Levels 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 adequately described and were obtained from a reliable source and were appropriate for use in this study.				
	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 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	Test conditions were conducive to maintenance of organism health				
		Cont	inued on nex	t page				

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continued from previous page							
Study Citation:	Battelle, (20	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis.					
Duration:	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:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064183						
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 Co	ntrol					
c	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures	C				
	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 Presenta	Metric 21.	Statistical Methods	High	Statistical methods were adequately described and were appropriate			
	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			
	Wette 25.	Explanation of Chexpected Outcomes	mgn				
Additional Comments:	None						
Overall Quality Determination High							

Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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; A Endocrine Dibutyl phth 10064183	Amphibian; <i>Xenopus laevis</i> ; Larvae alate (DBP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce		TT: 1		
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, CAS#, and structure.	
	Metric 2:	Test Substance Source	High	source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.	
	Metric 3:	Test Substance Purity	High	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.	
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated to tanks, and tanks were randomly 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 suitable and described in adequate detail. Exposure water-contact components included glass, stainless steel, and teflon.	
	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	High	The duration of exposure was suitable 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	High	Exposure concentrations were 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, and they were appropriate for use in this study.	
	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 numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.	
Domain 5: Outcom- A-	acamant				
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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continued from previous page							
Study Citation:	Battelle, (20	18). 21-d amphibian metamorphosis assay (AMA) of d	ibutyl phthalate with African clawed frog, xenopus laevis.			
Duration:	Overall Dura	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:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Endocrine	Endocrine					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	10064183						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	/ Variable Cou	ntrol					
Domain 0. Comounding	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	incure 17.	Design and Procedures	mgn	There were no reported differences unlong 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	TT' 1				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described and were appropriate.			
	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	Hıgh	There were no unexpected outcomes.			
Additional Comments:	ditional Comments: Thyroid gland histopathology was conducted in this study and evaluation included thyroid gland hypertrophy/atrophy, follicular cell hypertrophy, follicular cell hyperplasia, follicular lumen area, colloid quality and follicular cell height/shape.						
Overall Quality Determination			High				

Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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; A Behavioral Dibutyl phth	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae Behavioral Dibutyl phthalate (DBP)					
HERO ID:	10064183	Matria	Dating	Comments			
Domain 1: Test Substan	ce.	Metric	Kating	Comments			
Domain 1. Test Substan	Metric 1:	Test Substance Identity	High	Chemical was identified by name. CAS# and structure			
	Metric 2:	Test Substance Source	High	The source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.			
	Metric 3:	Test Substance Purity	High	Chemical purity 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 acceptable.			
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated to tanks, and tanks were randomly allocated to study groups.			
Domain 3 [,] Exposure Ch	aracterization						
Domain of Exposure on	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were suitable and described in adequate detail. Exposure water-contact components included glass, stainless steel, and Teflon.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups			
	Metric 9:	Administration 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 was suitable 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	High	Exposure concentrations were 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 and were appropriate for use in this study.			
	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 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	Test conditions 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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continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (20 Overall Dura Aquatic (free	 Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Larvae				
Health Outcome: Chemical: HERO ID:	Behavioral Dibutyl phth 10064183	Behavioral Dibutyl phthalate (DBP) 10064183				
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 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 and were appropriate.		
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High High	Data for exposure-related findings were presented for each treatment and control group There were no unexpected outcomes		
Additional Comments:	This form is	for the behavioral outcomes reported in the	text for the	definitive test.		
Overall Qualit	ty Detern	nination	High			

Study Citation: Duration: Exposure Route, Media, Path:	Battelle, (2018). 21-d amphibian metamorphosis assay (AMA) of dibutyl phthalate with African clawed frog, xenopus laevis. 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; Amphibian; <i>Xenopus laevis</i> ; Larvae Development/Growth Dibutyl phthalate (DBP) 10064183				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name, CAS#, and structure.	
	Metric 2:	Test Substance Source	High	The source and lot number of the test substance was reported, and a Certificate of Anal- ysis was provided.	
	Metric 3:	Test Substance Purity	High	Chemical purity 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 acceptable.	
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly allocated to tanks, and tanks were randomly allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
r in the r in the	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were suitable and described in adequate detail. Exposure water-contact components included glass, stainless steel, and Teflon.	
	Metric 8:	Consistency of Exposure	High	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	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit	
Domain 4: Test Organis	m				
e e e e e e e e e e e e e e e e e e e	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source and were appropriate for use in this study.	
	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 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	Test conditions 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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Dibutyl Phthalate

continued from previous page							
Study Citation:	Battelle, (20	18). 21-d amphibian metamorphosis assay ((AMA) of d	ibutyl phthalate with African clawed frog, xenopus laevis.			
Duration:	Overall Dura	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:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	10064183						
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	veie					
Domain 7. Data Present	Metric 21:	Statistical Methods	High	Statistical methods were adequately described and were appropriate.			
	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			
		1 1	0	1			
Additional Comments:	None						
Overall Quali	Overall Ouality Determination High						

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Study Citation:	Lee, S. K.,	Veeramachaneni, R., D.N. (2005). Subch	ronic exposur	e to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus
-	laevis frogs.	Toxicological Sciences 84(2):394-407.	-	
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 2	1 days	
Exposure Route,	Aquatic (fre	shwater), Aquatic (brackish); Water; Not	determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine
Media, Path:	exact uptake	route)		
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae		
Health Outcome:	Mechanistic	-Endocrine toxicity		
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	128004			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.
Domain 2: Test Design				
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative
		C C	C	control containing 0.01% DMSO.
	Metric 5:	Negative Control Response	High	The biological response of the negative controls was reported in the text under results.
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewal were conducted every M-W-F.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.
	Metric 11:	Number of Exposure Groups/	High	There were 5 exposure levels as well as a negative control and a solvent control. This was adequate to see a response
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.
Domain 4. Test Organis	m			
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.
		Cont	inued on nex	t page

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Environmental Hazard Evaluation

HERO ID: 128004 Table: 1 of 4

		conti	nued from p	revious page			
Study Citation:	Lee, S. K., laevis frogs.	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	Aquatic (freshwater), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine					
Media, Path:	exact uptake route)						
Taxa, Species, Age:	Vertebrate; Amphibian; Xenopus laevis; Larvae						
Health Outcome:	Mechanistic	Mechanistic-Endocrine toxicity					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	128004						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-plasma testosterone levels.			
	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	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 Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	Low	Data for the exposure related findings was reported in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.			
Additional Comments:	This portion mechanistic	of the evaluation was on the effect of D endocrine outcome was chosen.	BP on testos	sterone levels in Xenopus laevis. Plasma testosterone levels were measured, so th			

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater), Aquatic (brackish); 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 Dibutyl phthalate (DBP) 128004				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative control containing 0.01% DMSO.	
	Metric 5:	Negative Control Response	High	Mortality in the control groups were less than 10% throughout the exposure.	
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.	
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewal were conducted every M-W-F.	
	Weute 8.	Administration	Ingn	sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels as well as a negative control and a solvent control. This was adequate to see a response.	
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.	
Domain 4. Test Organia	~				
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.	
		Cont	inued on nex	at page	

Environmental Hazard Evaluation

HERO ID: 128004 Table: 2 of 4

		conti	nued from p	revious page			
Study Citation:	Lee, S. K., Jaevis frogs.	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (fre	shwater), Aquatic (brackish); Water; Not o	determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine			
Media, Path:	exact uptake	route)					
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	128004						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	Low	The mortality assessment was not described in the methods section.			
	Metric 18:	Consistency of Outcome	Low	How mortality was assessed was not reported.			
		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 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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	Medium	Mortality was just briefly described in text in the results section. Cumulative percent mortalities per treatment group were given there but no data was shown in any table or figure.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study authors did not report any unexpected outcomes. No variability was shown for mortality data.			
Additional Comments:	This evaluat	ion is for the mortality assessment during the	he study.				
Overall Qualit	ty Deterr	nination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater), Aquatic (brackish); 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 Reproductive/Teratogenic Dibutyl phthalate (DBP) 128004					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The DBP was identified by CASRN. The source was reported to be Sigma Aldrich, and the lot number was reported, but it		
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.		
		-	U	^ · ·		
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative control containing 0.01% DMSO.		
	Metric 5:	Negative Control Response	High	The biological response of the negative controls was reported in Figures 2,3, and 6, as well as in Tables 1 and 2.		
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewals were conducted every M-W-F.		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure levels as well as a negative control and a solvent control. This was adequate to see a response.		
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.		
Domain 4: Test Organisi	n					
	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.		
		Cont	inued on nex	t page		

Environmental Hazard Evaluation

HERO ID: 128004 Table: 3 of 4

		conti	nued from p	previous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Lee, S. K., Y laevis frogs. Overall Dura Aquatic (fre exact uptake Vertebrate; A Reproductiv Dibutyl phth	Veeramachaneni, R., D.N. (2005). Subchr Toxicological Sciences 84(2):394-407. ation: > 21 days; Exposure Duration: > 21 shwater), Aquatic (brackish); Water; Not of route) Amphibian; <i>Xenopus laevis</i> ; Larvae e/Teratogenic halate (DBP)	onic exposur days determined b	re to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus by study authors (i.e., chemical of interest in exposure water, but unable to determine
Domain	128004	Metric	Pating	Comments
Domain	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-histopathological changes in reproductive organs.
	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 to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7. Data Present	ation and Anal	vsis		
Domain / Data Present	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.
	Metric 22:	Reporting of Data	High	Data for exposure related findings including control results can be found in Tables 1 and 2 as well as in Figures 2,3 and 6. Other figures provided exposure related results only.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.
Additional Comments:	This portion effects on th	of the evaluation was on the effect of DBl e reproductive system in male frogs. Repro	P on spermat oduction was	togenesis in Xenopus laevis. Histopathological analysis was performed to observe the therefore selected as the outcome of interest.

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater), Aquatic (brackish); 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 Development/Growth Dibutyl phthalate (DBP) 128004				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.	
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative	
				control containing 0.01% DMSO.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 1.	
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.	
Domain 2: Exposure Ch	aractorization				
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewals were conducted every M-W-F.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure levels as well as a negative control and a solvent control. This was adequate to see a response.	
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate, indicat- ing the concentration was appropriate.	
Domain 4: Test Organisi	m Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.	
		Conti	nued on nex	xt page	

Environmental Hazard Evaluation

HERO ID: 128004 Table: 4 of 4

		conti	nued from p	revious page			
Study Citation:	Lee, S. K., laevis frogs.	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Aquatic (fre	Aquatic (freshwater), Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine					
Media, Path:	exact uptake route)						
Taxa, Species, Age:	Vertebrate; Amphibian; Xenopus laevis; Larvae						
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	128004						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-body weights/development/growth.			
	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 0. Comounding	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	infetite 19.	Design and Procedures	mgn	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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	High	Data for exposure related findings including control results can be found in Table 1.			
	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 development	of the evaluation was on the effect of D t/growth outcome was chosen.	BP on body	weight in Xenopus laevis. Body weights for each treatment were obtained, so the			
Overall Qualit	ty Detern	nination	High				

Study Citation:	Shen, O., W	Shen, O., Wu, W., Du, G., Liu, R., Yu, L., Sun, H., Han, X., Jiang, Y., Shi, W., Hu, W., Song, L., Xia, Y., Wang, S., Wang, X. (2011). Thyroid disruption by Di-n-butyl phthalate (DBP) and mono-n-butyl phthalate (MBP) in Xenopus laevis. PLoS ONE 6(4):e19159				
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: No	ot-reported			
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:	Vartabrata: /	mphihian: Vananus Laguis: Lagua				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	787926					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CAS number.		
	Metric 2:	Test Substance Source	Low	The source was reported to be Tokyo Kasei, however, it was not reported if the DBP was analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be >99%.		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	A negative solvent control was reported to be used in this study.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 2, and was adequate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the tadpoles were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	Stock solution was made by adding 250mg/L of DBP into DMSO. The stock solution		
	inedic /:	Preparation	mgn	was then diluted to the proper test concentration by adding to dechlorinated water in the appropriate amount.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 21 days with test solutions renewed every two days. All exposure concentrations had 20 tadpoles in 5L of test solution.		
	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:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 21 days, which appeared adequate for a re- sponse.		
	Metric 11:	Number of Exposure Groups/	Medium	There were only three exposure levels, but this appeared adequate for a dose response. The spacing was appropriate for the study		
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO was used as a vehicle solvent in this study at a concentration of 0.005%.		
Domain 1: Test Organia						
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	Spawning adults were reported to be from Nasco in the United States. Tadpoles were obtained from these spawners and were placed into the test system at stage 51 (14-16d).		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the tadpoles were acclimated for any length of time.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 20 tadpoles per test chamber, but the number of replicates per concentration was not reported.		

Continued on next page ...

HERO ID: 787926 Table: 1 of 2

continued from previous page				
Study Citation: Duration: Exposure Route, Media Path:	Shen, O., Wu, W., Du, G., Liu, R., Yu, L., Sun, H., Han, X., Jiang, Y., Shi, W., Hu, W., Song, L., Xia, Y., Wang, S., Wang, X. (2011). Thyroid disruption by Di-n-butyl phthalate (DBP) and mono-n-butyl phthalate (MBP) in Xenopus laevis. PLoS ONE 6(4):e19159. Overall Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae Development/Growth Dibutyl phthalate (DBP) 787926			
Domain		Metric	Rating	Comments
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	High	Tadpoles were fed Nasco Frog Brittle and were kept at 22 C with a 12L:12D photope- riod.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-development/growth.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Tadpoles were assessed daily for their developmental stages. On day 22, stage, body length, interocular distance, and body-to-tail length ratio.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There weren't any reported 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 or health outcomes unrelated to exposure.
Domain 7: Data Present	ation and Anal	lysis		
	Metric 21:	Statistical Methods	High	ANOVA and Duncan's multiple comparison test were used for statistical analysis.
	Metric 22:	Reporting of Data	High	Data for the control response and the dose response can be found in Table 2 and are adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability is reported in Table 2.
Additional Comments:	This portion of the study is on the effect of DBP on the stage, length, intraocular distances, and the body-to-tail length ration. Development/growth was selected as the outcome of interest. Tadpoles were kept in aquariums until Stage 57. A time period was not given for this, thus the "not reported" study duration.Nieuwkoop PD, Faber J (1994)Normal Table of Xenopus laevis (Daudin): ASystematical and Chronological Survey of the Development from the FertilizedEgg till the End of Metamorphosis; Garland Publishing: New Yorkwas cited as the reference for staging the tadpoles in the methods.			
Overall Qualit	ty Deterr	nination	High	
HERO ID: 787926 Table: 2 of 2

Study Citation: Duration: Exposure Route,	Shen, O., W by Di-n-buty Overall Dura Aquatic (free	u, W., Du, G., Liu, R., Yu, L., Sun, H., Ha /l phthalate (DBP) and mono-n-butyl phtha ation: 11 - 21 days; Exposure Duration: No shwater); Water; Not determined by study	n, X., Jiang, alate (MBP) i ot-reported authors (i.e.,	Y., Shi, W., Hu, W., Song, L., Xia, Y., Wang, S., Wang, X. (2011). Thyroid disruption n Xenopus laevis. PLoS ONE 6(4):e19159. chemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mechanistic Dibutyl phth 787926	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics-Endocrine toxicity Dibutyl phthalate (DBP) 787926					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT: 1				
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The DBP was identified by CAS number. The source was reported to be Tokyo Kasei, however, it was not reported if the DBP was analytically verified			
	Metric 3:	Test Substance Purity	High	The purity was reported to be $>99\%$.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	A pagative solvent control was reported to be used in this study			
	Metric 5:	Negative Control Response	High	The negative solvent control was reported to be used in this study. The negative control response was reported in Figures 2 and 3, and was adequate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the tadpoles were allocated into study groups.			
	, . , .						
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	Stock solution was made by adding 250mg/L of DBP into DMSO. The stock solution was then diluted to the proper test concentration by adding to dechlorinated water in the appropriate amount.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 21 days with test solutions renewed every 2 days. All exposure concentrations had 20 tadpoles in 5L of test solution.			
	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:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 21 days, which appeared adequate for a re- sponse.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only 3 exposure levels, but this appeared adequate for a dose response. The spacing was appropriate for the study.			
	Metric 12:	Testing at or Below Solubility Limit	High	DMSO was used as a vehicle solvent in this study at a concentrations of 0.005%			
Domain 4. Test Organis							
Domain 1. Test organis	Metric 13:	Test Organism Characteristics	High	Spawning adults were reported to be from Nasco in the United States. Tadpoles were obtained from these spawners and were placed into the test system at stage 51 (14-16d).			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the tadpoles were acclimated for any length of time.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 20 tadpoles per test chamber, but the number of replicates per concentration was not reported.			
				and the second			

Domain 5: Outcome Assessment

HERO ID: 787926 Table: 2 of 2

		conti	nued from p	previous page			
Study Citation:	Shen, O., W by Di-n-buty	Shen, O., Wu, W., Du, G., Liu, R., Yu, L., Sun, H., Han, X., Jiang, Y., Shi, W., Hu, W., Song, L., Xia, Y., Wang, S., Wang, X. (2011). Thyroid disruption by Di-n-butyl phthalate (DBP) and mono-n-butyl phthalate (MBP) in Xenopus laevis. PLoS ONE 6(4):e19159					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: Not-reported					
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; A	Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell sign	naling/functi	on-Epigenetics-Endocrine toxicity			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	787926						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were fed Nasco Frog Brittle and were kept at 22 C with a 12L:12D photope- riod.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–gene expression after exposure.			
Metric 18: Consistency of Outcome High Details of the outcome assessment protocol were reported, and outco 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 conditions for 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 in animal attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	ANOVA and Duncan's multiple comparison test were used for statistical analysis. Chi- square was used for the portion of the study pertaining the methylation.			
	Metric 22:	Reporting of Data	High	Data for the control response and the dose response can be found in Figures 2 and 3 and in Table 3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability is reported in the figures and tables.			
Additional Comments:	S: This portion of the study is on the effect of DBP on gene expression relating to the the thyroid and endocrine system as well as methylation. Mechanistic outcomes were selected for this reason. Tadpoles were kept in aquariums until Stage 57. A time period was not given for this, thus the "not reported" study duration.Nieuwkoop PD, Faber J (1994)Normal Table of Xenopus laevis (Daudin): ASystematical and Chronological Survey of the Development from the FertilizedEgg till the End of Metamorphosis; Garland Publishing: New Yorkwas cited as the reference for staging the tadpoles in the methods.						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Cruciani, V., Brachionus o	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 calveiflorus, Ecotoxicology 25(1):192-200.					
Duration: Exposure Route, Modia Poth	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:	Invertebrate; Reproductive	nvertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Brachionus calyciflorus</i> ; Pallas; Larvae Reproductive/Teratogenic					
Chemical: HERO ID:	Dibutyl phth 3070931	alate (DBP)					
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	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 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 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 initial exposure administration were reported but details of exposure admin- istration past 48 hours was 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 emptied into a glass petri dish for counting males and females, but 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 four exposure groups for DBP and five replicates were run for each concen-			
	14.1.10	Spacing of Exposure Levels		tration.			
	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 Organise	m						
2 onium 1. Tost Organisi	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
		Contin	nued on next pa	nge			

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 3070931 Table: 1 of 1

		contin	ued from previ	ious page	
Study Citation: Duration: Exposure Route, Media Path:	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: Health Outcome: Chemical: HERO ID:	Invertebrate: Reproductiv Dibutyl phth 3070931	; Other Invertebrate (e.g., sea urchins, ciliate e/Teratogenic nalate (DBP)	s, rotifers); Brac	chionus calyciflorus; Pallas; Larvae	
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 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 housing, environmental conditions and food were conducive to maintenance of health. It was reported that the population growth rate at 48 h in the control was 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 fertil- ized female and detached fertilized eggs. In the paper and 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	/ Variable Co	ntrol			
e	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	ation and Anal	vsis			
	Metric 21: Metric 22: Metric 23:	Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Statistical methods were clearly described. Data for exposure-related findings were presented for each treatment and control group There were no unexpected outcomes.	
Additional Comments:	DBP exposu at 2mg/L aft	re negatively affected asexual reproduction er 96 hours.	in rotifers at the	highest concentration tested (2mg/L) at 48 and 72 hours and sexual reproduction	
Overall Qualit	v Deterr	nination	Medium		

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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 calveiflorus Pallas. Aquatic Ecology 43(2):395-402						
Duration:	Overall Dura	ation: $4 - 10$ days; Exposure Duration: $4 - 10$ d	days				
Exposure Route, Media Path:	Aquatic (free	shwater); Water; Not determined by study auti	hors (1.e., chen	nical of interest in exposure water, but unable to determine exact uptake route)			
Taxa. Species. Age:	Invertebrate:	Invertebrate; Arthropods; Brachionus calvciflorus; Juvenile					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1336226						
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 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 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.			
	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	Low	Concentrations exceeded solubility, but with solvents they were at an appropriate level aided in dissolution.			
Domain 4. Toot Orregies							
Domain 4: Test Organisi	III Matric 12:	Test Organism Characteristics	Madium	Ease wars callected from Lake linghy and cultured in the leb. There are minor records			
	wieure 15:	Test Organism Characteristics	weduiii	tions 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:	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

HERO ID: 1336226 Table: 1 of 2

		contin	ued from previo	bus 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					
	calyciflorus	Pallas. Aquatic Ecology 43(2):395-402.					
Duration:	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:	Invertebrate; Arthropods; Brachionus calyciflorus; Juvenile						
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1336226						
Domain		Metric	Rating	Comments			
	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					
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	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 Qualit	ty Detern	nination	Medium				

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 Brachic 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; Mortality Dibutyl phth 1336226	nvertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile Mortality Dibutyl phthalate (DBP) 1336226					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High High	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 reported as $>=97\%$			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control groups			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was adequate			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups			
Damain 2. East and Ch							
Domain 5: Exposure Ch	Matria 7:	Experimental System/Test Media	High	The experimental system and methods for proportion of test media years described in			
	Methe 7.	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:	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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility but solvents at an appropriate level aided in dissolu- tion			
Domain 4. Test Organiss	m						
Domain 4. Test Organish	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations source of test organisms that are unlikely to have a sub- stantial impact on results. Organisms were collected in the sediment of Lake Jinghu and cultured in the lab.			
	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			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health			
Continued on next page							

Environmental Hazard Evaluation

HERO ID: 1336226 Table: 2 of 2

		contin	ued from previ	ous 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					
Duration:	Overall Dura	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:	Invertebrate: Arthropods: Brachionus calvciflorus: Juvenile						
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1336226						
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 condition			
		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 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						

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: Exposure Route, Media, Path:	Overall Dura Aquatic (fres	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 plumosus; Larvae	e			
Health Outcome:	Immobilizati	on				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	813673					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce		T			
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	lest Substance Source	Low	not reported if the DBP was reported to be Aldrich Company in St. Louis, MO. It was not reported if the DBP was analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DBP was 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	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 2. Evenagues Ch	anastanization					
Domain 5: Exposure Ch	Metric 7	Experimental System/Test Media	Low	Little information was provided on the preparation of the test media		
	Wette 7.	Preparation	LOW	Entre information was provided on the preparation of the est media.		
	Metric 8:	Consistency of Exposure	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	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 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 Organise	m					
Domain 4. Test Organisi	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 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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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 813673 Table: 1 of 1

		0	ontinued from previous	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 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 plumosus; Larvae					
Health Outcome:	Immobilization					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	813673					
Domain		Metric	Rating	Comments		
Domain	Metric 15:	Number of Organisms and	Low	The number of test organisms per test chamber and the number of replicates was not		
	Wieute 15.	Replicates per Group	Low	reported, though this may have been included in the citation for methodology.		
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. 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: 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 or health outcomes unrelated to exposure.		
Domain 7. Data Present	ation and Anal	lysis				
Domain 7. Dua i losona	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		
			0	2.		
Additional Comments:	This portion selected as the selected selected as the selected selected as the selected selec	This portion of the evaluation was on the acute toxicity of DBP on C. pulmosus. The study reported assessing immobilization as the outcome, so that was selected as the outcome for the evaluation. The study received an unacceptable ranking due to the lack of reporting on exposure groups and spacing.				
Overall Quali	ty Deterr	nination	Uninformative	e		

Study Citation: Duration: Exposure Route, Media, Path:	Streufort, J. Overall Dura Aquatic (free	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: Chemical:	Invertebrate; Mortality Dibutyl phth	Arthropods; <i>Chironomus plumosus</i> ; Larvae alate (DBP)	;					
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	Source was listed from Monsanto but not analytically verified.				
	Metric 3:	Test Substance Purity	Low	No purity was reported.				
Domain 2. Test Design								
2 ciliani 21 1000 2 colgi	Metric 4:	Negative Controls	Low	Ethanol was listed for solvent controls, however, authors reported using ethanol con- centrations at higher than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of compounds.				
	Metric 5:	Negative Control Response	Low	Control responses are 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							
	Metric 7:	Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal, with morality 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 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 are reported. Range finding tests were not described.				
	Metric 12:	Testing at or Below Solubility Limit	Low	The LC50 values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since they were not reported.				
Domain 4. Test Oreania								
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 concentrations but did not report the level of replication for each treatment.				

Domain 5: Outcome Assessment

Dibutyl Phthalate

		contir	nued from previo	us page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Streufort, J. Overall Dura Aquatic (free Invertebrate; Mortality	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 Mortality				
Chemical: HERO ID:	Dibutyl phth 1332972	alate (DBP)				
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Medium	Dissolved oxygen, temperature, and photoperiod were reported for the acute bioassays.		
	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: Confounding	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 outcomes.		
Domain 7: Data Present	ation 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 Quali	ty Detern	nination	Medium			

Dibutyl Phthalate

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: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 1332972	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae Mortality Dibutyl phthalate (DBP) 1332972					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Nomenclature referenced on page 13/62. No CAS or structure listed.			
	Metric 2:	Test Substance Source	Low	Source was listed from Monsanto but not analytically verified.			
	Metric 3:	Test Substance Purity	Low	No purity reported.			
Domain 2: Tast Dasign							
Domain 2. Test Design	Metric 4:	Negative Controls	Low	Ethanol listed for solvent controls, however, authors report using ethanol concentrations at higher than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of compounds.			
	Metric 5:	Negative Control Response	Low	Control responses are not reported for acute toxicity bioassays.			
	Metric 6:	Randomized Allocation	Low	No reporting on how animals were allocated to treatment concentrations.			
Domain 3: Exposure Ch	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	Medium High Low High Low Low	The acute toxicity bioassays were conducted as static non-renewal, with morality as- sessed at 24 and 48 hours. exposure administration appeared consistent among treatments and control. The acute bioassay concentrations were not analyzed and LC50 concentrations are re- ported as nominal. The duration (48 hr) is appropriate. Non of the treatment concentrations for the acute bioassays are reported. Range finding tests were not described. The LC50 values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since they were not reported.			
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 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 did not report the level of replication for each treatment.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the acute bioassays.			
		Contin	nued on next pa	ge			

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Dibutyl Phthalate

		conti	nued from previo	bus page		
Study Citation: Duration: Exposure Route, Media, Path: Toxo, Species Age:	Streufort, J. Overall Dura Aquatic (free	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)				
Health Outcome:	Mortality	Mortality				
Chemical: HERO ID:	Dibutyl phth 1332972	alate (DBP)				
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: Confounding	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	No information to suggest differences among groups related to health outcomes.		
Domain 7: Data Present	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., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.						
D (1	Environmen	tal Research 6(1):84-90.	10.1				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 days	anatin averagene water het unable to determine event untelle soute)			
Exposure Koule, Modia Path	Aquatic (fre	shwater); water; Not determined by study	authors (i.e., chemical of inte	erest in exposure water, but unable to determine exact uptake route)			
Tava Snecies Age	Invertebrate	Arthropods: Chironomus plumosus: Larve	ae -				
Health Outcome:	ADME (bio	ADME (biotransformation)					
Chemical:	Dibutyl phth	alate (DBP)					
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							
6	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		-				
	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
	Metric 8.	Preparation Consistency of Exposure	Low	test concentrations. Few details of exposure administration were reported, but exposures were administered			
	Metric 8.	Administration	Low	consistently across study groups			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.			
		Concentration	8				
	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.			
		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.			
	Mate 15	Conditions	T				
	Metric 15:	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	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.			
			Continued on next page				

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Dibutyl Phthalate

HERO ID: 1334646 Table: 1 of 1

Study Citation							
Study Charlon:	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.						
	Environment	Invironmental Research 6(1):84-90.					
Duration:	Overall Dura	tion: 4 - 10 days; Exposure Duration: 4 - 10 c	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:	Invertebrate; Arthropods; Chironomus plumosus; Larvae						
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Dibutyl phth	alate (DBP)					
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 wer			
		Assessment		provided.			
Domain 6: Confounding	/Variable Cor	atral					
Domain 0. Comounding	Metric 10:	Confounding Variables in Test	Low	The study did not provide arough information to allow a comparison of anyironmenta			
	Wieule 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			
	Metrie 20.	Succines Chickaed to Exposure	Weddulli	attrition.			
Domain 7: Data Presenta	tion 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.			
		Enplanded of energeolog outcomes					
Additional Comments:	None						

Study Citation: Duration: Exposure Route, Media Path:	Streufort, J. Overall Dura Aquatic (frea	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: Health Outcome: Chemical: HERO ID:	Invertebrate: Mortality Dibutyl phth 1332972	; Arthropods; <i>Chironomus plumosus</i> ; Larvae nalate (DBP)	2					
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 were listed.				
	Metric 2:	Test Substance Source	Low	Source was listed from Monsanto but 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							
I	Metric 7:	Experimental System/Test Media Preparation	High	The chronic exposure were 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	Exposure administration appeared consistent among treatments and 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:	Concentration 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 all below the published solubility value published in the final scope for DBP (11.2 mg/l).				
Domain 4: Test Organis	m							
C C	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:	Number of Organisms and Replicates per Group	Low	Authors began each treatment and control group with 100 1st instar larvae for the chronic emergence bioassay. The replication and housing groups for this work was not well described.				
Domain 5: Outcome As	sessment			wen described.				

Dibutyl 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 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:** Mortality **Chemical:** Dibutyl phthalate (DBP) **HERO ID:** 1332972 Domain Metric Rating Comments Metric 16: Adequacy of Test Conditions Medium Dissolved oxygen, temperature, and photoperiod were reported for the chronic exposures. Metric 17: Outcome Assessment Methodology Medium No significant differences in emergence were observed for the chronic exposures. 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 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	Call, D. J., C Parkerton, T exposures. E Overall Dura Aquatic (free	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 Aquatic (freehwater): Sediment: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route).							
Media, Path:	Aquatic (IIe.	quare (reshwater), seament, not determined by study autions (i.e., enernear or interest in exposure water, but unable to determine exact uptake foule)							
Taxa, Species, Age:	Invertebrate:	Arthropods: Chironomus tentans: Larvae							
Health Outcome:	Developmen	t/Growth							
Chemical:	Dibutyl phthalate (DBP)								
HERO ID:	679311								
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ice								
	Metric 1:	Test Substance Identity	High	The chemical was identified as a "single isomer" and the identify, including CASRN referenced in an outside paper, 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 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	Biological response of control groups was appropriate as shown in Table 4.					
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.					
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 test beakers were described in detail.					
	Metric 8:	Consistency of Exposure Administration	High	Exposure consistency was reported and consistent among different DBP treatments and controls.					
	Metric 9:	Measurement of Test Substance	High	Concentrations were measured using HPLC as described in the methods and cited reference (Call et al 2001).					
	Metric 10:	Exposure Duration and Frequency	High	The duration (10-day exposure) was appropriate for the experimental design.					
	Metric 11:	Number of Exposure Groups/	Medium	Exposure groups were acceptable and spanned five concentrations per test species in					
		Spacing of Exposure Levels		addition to the control. Nominal doses were unclear, however measured doses were reported in sediment and pore water.					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via sediment.					
Domain 4. Test Organia	m								
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of test organisms was not reported					
	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 DRP utilized three replicates of five different concentrations with 10 organ-					
	mente 15.	Replicates per Group	meanni	isms per beaker; three sediment control replicates with 10 test organisms per beaker; and three silica sand control replicates with 10 test organisms per beaker.					

Environmental Hazard Evaluation

HERO ID: 679311 Table: 1 of 2

		conti	nued from p	revious page		
Study Citation:	Call, D. J., C Parkerton, T exposures. E	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	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; Chironomus tentans; Larvae Development/Growth					
Health Outcome:	Developmen	lt/Growth				
UEDO ID:	CIDUCTION	lalate (DBP)				
HERO ID:	0/9311					
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, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions were described in Table 2.		
	Metric 17:	Outcome Assessment Methodology	High	Sediment was sieved, survivors collected, dried, and weighed.		
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted 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 the study groups in environmental conditions or other factors that could influence the 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 4. Results were represented as aver- age dry weight per individual.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported, but results suggest no excessive variability within repli- cates.		
Additional Comments:	None					
Overall Quality Determination			High			

HERO ID: 679311 Table: 2 of 2

Study Citation:	Call, D. J., C Parkerton, T.	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	115.			
Exposure Route,	Aquatic (free	shwater); Sediment; Not determined by stu	udy 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:	Dibutyl phth	alate (DBP)					
HERO ID:	679311						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified as a "single isomer" and the identify, including CASRN referenced in an outside paper, Call et al 2001.			
	Metric 2:	Test Substance Source	High	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 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	Biological response of control groups was appropriate as shown in Table 4.			
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.			
Domain 3: Exposure Ch	aracterization						
Domain 9: Exposure on	Metric 7:	Experimental System/Test Media Preparation	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 Administration	High	Exposure consistency reported and consistent among different DBP treatments and controls.			
	Metric 9:	Measurement of Test Substance	High	Concentrations measured using HPLC as described in methods and cited reference (Call et al 2001).			
	Metric 10:	Exposure Duration and Frequency	High	Duration (10 day exposure) was appropriate for experimental design.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were acceptable and spanned 5 concentrations per test species in addi- tion to control; nominal doses unclear however measured doses reported in sediment and pore water.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via sediment.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	Source of test organism not reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation of test organisms prior to exposure not reported.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized 3 replicates of five different concentrations with 10 organisms per beaker and three sediment control replicates with 10 test organisms per beaker and three silica sand control replicates with 10 test organisms per beaker.			

Domain 5: Outcome Assessment

Environmental Hazard Evaluation

HERO ID: 679311 Table: 2 of 2

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route	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 Aquatic (freshwater): Sediment: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route).						
Media, Path:	Aquatic (IIC	require (neshwater), sediment, rot determined by study additis (ne., enermed of merest in exposure water, but anable to determine exact aplane route)					
Taxa. Species. Age:	Invertebrate	Invertebrate; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Mortality						
Chemical:	Dibutyl phthalate (DBP)						
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, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions described in Table 2.			
	Metric 17:	Outcome Assessment Methodology	Medium	Survivor count determined after the 10 day exposure but not reported as percent mortal- ity.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted at conclusion of 10 day exposure.			
Domain 6: Confounding	v / Variable Co	ntrol					
2 011111 01 00110411411	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	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	tation and Anal	vsis					
	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	Survival data reported in Table 4 and LC50 values shown in Figure 1 and Table 6.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability not reported but results suggest no excessive variability within replicates.			
Additional Comments:	None						
Ownell Orall	ty Doto-		II:ab				
Overall Quali	iy Deterr	iiiiauoli	nign				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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. 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 tentans</i> ; Larvae Development/Growth Dibutyl phthalate (DBP) 679312					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	Chemical was identified by name, no CASRN or structure.		
	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 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 Ch	aracterization 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	High	exposures were administered consistently across study groups.		
	Metric 9:	Administration 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 exposure frequency were 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 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	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 Assessment

Environmental Hazard Evaluation

HERO ID: 679312 Table: 1 of 2

		conti	nued from p	previous page			
Study Citation:	Call, D. J., J F., Reiley, M Environmen	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.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	l0 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:	Invertebrate	Invertebrate; Arthropods; Chironomus tentans; Larvae					
Health Outcome:	Developmen	tt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	679312						
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	High	outcomes were assessed consistently across study groups			
		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 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	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:	None						
Overall Qualit	ty Deterr	nination	High				

HERO ID: 679312 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media, Path:	Call, D. J., I F., Reiley, M Environmen Overall Dura Aquatic (fre	 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. 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:	Invertebrate: Mortality Dibutyl phth	; Arthropods; <i>Chironomus tentans</i> ; Larvae nalate (DBP)					
Domain	079312	Metric	Rating	Comments			
Domain 1: Test Substan	ce		8				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name only. There was no CASRN or structure reported.			
	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: Tast Dasian							
Domain 2. Test Design	Metric 4.	Negative Controls	High	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 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 Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.			
	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	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	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4. Total Orto							
Domain 4: Test Organis	III Matria 12.	Test Organism Characteristics	Ulah	The test arranisms were adaptately described and were obtained from a reliable			
	Metric 14:	A colimatization and Protroctment	rign Liab	All protocotmont conditions were the same for control and evenesid eventions.			
	Metric 14:	Conditions	пign	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 As	sessment	A degree of Test Conditions	High				
	Metric 16:	Adequacy of rest 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.			
		Cont	inued on ney	xt page			

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Environmental Hazard Evaluation

HERO ID: 679312 Table: 2 of 2

		contr	nued from p	revious page				
Study Citation:	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.							
Duration:	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:	Invertebrate; Arthropods; Chironomus tentans; Larvae							
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	679312							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
Domain & Canfaundina	/ Variable Co	ntrol						
Domain 6: Contounding	/ variable Col	nuon Conformatina Variables in Test	TT: -1-					
	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				
	Wietrie 20.	Outcomes Onrelated to Exposure	Ingn	There were no unreferees among groups that could influence the outcome assessment.				
Domain 7: Data Presenta	ation and Anal	vsis						
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.				
	Metric 22:	Reporting of Data	Medium	Mean 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.				
	The LC50 of DBP for Chironomus was reported as 2.64 mg/L.							

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus tentans; Not Ap	plicable (e.g.	, fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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							
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	Medium	The study reported that organisms were randomly allocated into study groups.			
Damain 2: Error over Ch							
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Media	Uiah	The eventimental system and methods for momentian of test modio years described in			
	Metric 7.	Preparation	rigii	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	High	Exposure concentrations were measured using appropriate analytical technologies and			
		Concentration		methods.			
	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 suitable for a dose			
	M 10	Spacing of Exposure Levels	TT: 1	response.			
	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 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.			
	Matria 15.	Conditions	Madium	These wars 10 encentions not serilizate with two serilizates used			
	Metric 15.	Replicates per Group	Wiedium	There were to organisms per replicate with two replicates used.			
Domain 5: Outcome Ass	sessment		TT' 1				
	Metric 16:	Adequacy of fest 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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Dibutyl Phthalate

HERO ID: 7325945 Table: 1 of 4

		····contin		n evious page			
Study Citation:	Lake Superio	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 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:	Invertebrate;	Arthropods; Chironomus tentans; Not App	licable (e.g.	., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
		Assessment					
Domain 6: Confounding	/ 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	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 shown for each treatment and control group.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
	None						

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 7325945 Table: 2 of 4

Study Citation: Duration: Exposure Route, Media Path:	 Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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; <i>Chironomus tentans</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality						
Health Outcome:							
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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							
in the second se	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 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 methods for preparation of test media were described in			
	Wette 7.	Preparation	mgn	adequate detail.			
	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 using appropriate analytical technologies and methods			
	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 suitable for a dose			
	Medic II.	Spacing of Exposure Levels	mgn	response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.			
Domain 4. Test Organis	m						
2 chian 1. 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.			
		Conditions	8	· · · · · · · · · · · · · · · · · · ·			
	Metric 15:	Number of Organisms and	Medium	There were 10 organisms per replicate with two replicates 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 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	6				

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 2 of 4

continued from previous page								
Study Citation:	Lake Superio	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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; Chironomus tentans; Not App	plicable (e.g.	, fungi or algae studies) or Not Reported				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	7325945							
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	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	Medium	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the tables.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.				
Additional Comments:	None							
Overall Quality Determination			High					

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HERO ID: 7325945 Table: 3 of 4

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)							
Taxa, Species, Age:	Invertebrate;	Invertebrate: Arthropods: Chironomus tentans; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality							
Chemical:	Dibutyl phthalate (DBP)							
HERO ID:	7325945							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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								
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.				
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into 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 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 appropriate analytical technologies and methods.				
	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	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 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	High	All pretreatment conditions were the same for control and exposed organisms.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 organisms per replicate with two replicates 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 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	0	,,				

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 3 of 4

continued from previous page								
Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (free	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 7325945							
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 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 shown for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.				
Additional Comments:	None							
Overall Quality Determination		High						

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HERO ID: 7325945 Table: 4 of 4

Study Citation: Duration: Exposure Route, Media. Path:	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age:	Invertebrate;	Arthropods; Chironomus tentans; Not Ap	plicable (e.g.	, fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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							
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.			
	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	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 appropriate analytical technologies and methods.			
	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	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 4: Test Organis	n						
<i>0</i>	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	There were 10 organisms per replicate with two replicates used.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
Domain 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:	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	, ,, ,, ,,			

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 4 of 4

continued from previous page								
Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (free	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 7325945							
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 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 shown for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.				
Additional Comments:	None							
Overall Quality Determination		High						

Study Citation: Duration: Exposure Route, Media, Path:	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 aquatiorganisms. 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: Health Outcome: Chemical:	Invertebrate; Immobilizati Dibutyl phth	Arthropods; <i>Daphnia magna</i> ; Not Applic ion alate (DBP)	able (e.g., fur	ngi or algae studies) or Not Reported		
HERO ID:	1321996					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		Ŧ			
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low Low	The test substance nomenclature was reported without a CASRN. The source was reported as provided by a manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data were reported.		
	Metric 3:	Test Substance Purity	High	The substance was at least 95% pure.		
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	An allocation method was not reported.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to pre- vent 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 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 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 water solubility.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation 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	· · ·				

HERO ID: 1321996 Table: 1 of 1

		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. H	rganisms. 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	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; Not Applica	able (e.g., fur	ngi or algae studies) or Not Reported					
Health Outcome:	Immobilizat	ion							
Chemical:	Dibutyl phth	alate (DBP)							
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	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 e						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.					
Domain 7. Data Present	ation and Anal	veic							
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Statistical methods were performed and described					
	Metric 22:	Reporting of Data	Medium	Only treatment and points were reported					
	Metric 22.	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported					
	wienie 23.	Explanation of Onexpected Outcomes	Ingn						
Additional Comments:	None								
Overall Qualit	ty Deterr	nination	High						
Study Citation:	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to Daphnia magna (final report).								
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Duration: Exposure Route,	Overall Dura Aquatic (free	ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study at	n: 0 - 4 days (0-96h) uthors (i.e., chemical of int	terest in exposure water, but unable to determine exact uptake route)					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 1316223	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Mortality Dibutyl phthalate (DBP)							
Domain		Metric	Rating	Comments					
Domain 1: Test Substan	ce		8						
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Medium Low	The chemical is identified by name and CASRN, but no other verification is provided. 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	Metric 1:	Negative Controls	High	Study authors reported using an appropriate concurrent pagative control group (i.e. all					
	Meure 4.	Regative controls	Ingn	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								
Domain 3. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Uninformative	The experimental system and/or test media preparation methods were adequately reported but did not account for physical-chemical properties, specifically the low solubility in the test medium and rapid degradation rate. The authors measured the test concentrations throughout the test, but the rate of degradation was very high, resulting in a final test concentration below the limit of detection of the measurement device. As a result, it cannot be determined whether the lack of mortality observed in the study is the result of the toxicity of the test material, or the lack of test material in the exposure vessel. Furthermore, because the final concentration is reported as a non definitive value ($<$), a mean-measured concentration cannot be determined.					
	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 measured using appropriate analytical technologies and methods. Analytical technologies used were highly sensitive (GC-MS), but not sensitive enough to measure the concentration in the corroborative test.					
	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 and insufficient to capture an endpoint.					
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	Exposure concentrations were seemingly above the water solubility limit in the initial test. The authors reported that the initial test featured a film that captured and killed a significant number of the daphnia. A solvent should have been used.					
		(Continued on next page						

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Dibutyl Phthalate

Evaluation

HERO ID: 1316223 Table: 1 of 1

		c	ontinued from previous	page		
Study Citation: Duration: Exposure Route, Media. Path:	Bionomics,, Overall Dura Aquatic (fres	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to Daphnia magna (final report). 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; Arthropods; <i>Daphnia magna</i> ; Juvenile Mortality					
Chemical: HERO ID:	Dibutyl phth 1316223	alate (DBP)				
Domain		Metric	Rating	Comments		
Domain 4: Test Organist	n					
	Metric 13: Metric 14:	Test Organism Characteristics Acclimatization and Pretreatment Conditions	Low Low	The source (and sex if relevant) of the test animals was not reported. 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	Five daphnia/replicate is far lower than the guideline required 20 daphnia/replicate.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to maintenance of health, and biomass loading was appropriate.		
	Metric 17:	Outcome Assessment Methodology	Uninformative	Significant deficiencies in the reported outcome assessment methodology were iden- tified. 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 the first definitive test, the authors report that a film of insoluble test material formed on the surface that entrapped some of the test organisms and caused mortality. In the follow-up, corroborative test, another method was used to reduce the occurrence of the film, but the test material completely degraded by the end of that test, rendering it impossible to draw a conclusion about the toxicity of the substance, as no mortality was observed.		
	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 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	Uninformative	A film of insoluble test material caused mortality in the test organisms.		
Domain 7: Data Presenta	ation and Anal	vsis				
	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.		
		(Continued on next page .			

Dibutyl Phthalate

	c	ontinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Springborn (1984). Acute toxicity of fourted Overall Duration: 0 - 4 days (0-96h); Exposure Duration Aquatic (freshwater); Water; Not determined by study au Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Mortality Dibutyl phthalate (DBP) 1316223	en phthalate esters to Daj a: 0 - 4 days (0-96h) athors (i.e., chemical of i	ohnia magna (final report). nterest in exposure water, but unable to determine exact uptake route)		
Domain	Metric	Rating	Comments		
	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. In addition, there was significant, non-dose dependent mortality observed in the lower concentrations of the initial test that was not explained.		
Additional Comments:	This study result should be interpreted with caution. This test was comprised of two 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 completely degraded by the end of the test, and no mortality was observed.				
0 110 11					

Overall Quality Determination

Uninformative

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 Durati):380-386. on: 0 - 4 davs	(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:				
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Immobilizati			
Chemical:	Dibutyl phth	alate (DBP)		
	3730702			
Domain	20	Metric	Rating	Comments
Domain 1: Test Substanc	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
			2011	the performing laboratory.
	Metric 3:	Test Substance Purity	Medium	It was mentioned that chemicals used were analytical grade.
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.
	, . <i>.</i> .			
Domain 3: Exposure Cha	Matric 7:	Experimental System/Test Media	Madium	Test modio proportion mothods were reported but did not provide the measures taken
	Weule 7.	Preparation	Weddulli	to minimize loss of test substance before and during the exposure. Concentration of test substance was 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 Concentration	High	Exposure concentrations were measured using HPLC. The percent recovery of spiked test substance was within 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	Seven 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 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 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 ten daphinds per test vessel and they were tested in triplicates.
		Cont	tinued on nex	at page

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Dibutyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Huang, B., I of Environm	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					
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:	Invertebrate	; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Immobilizat	ion					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5750702						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	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	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		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	tation 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 analysi	Data analysis methods were not provided. EC 50 values were given without confidence intervals.					

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path:	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. 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 Dibutyl phth 789536	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Larvae Immobilization Dibutyl phthalate (DBP)					
Domain	10,550	Metric	Rating	Comments			
Domain 1: Test Substan	ice	Wette	Ruting	connicits			
	Metric 1:	Test Substance Identity	High	The test substance was identified definitively (nomenclature, CASRN, and structure were reported).			
	Metric 2:	Test Substance Source	High	DBP 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 >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 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						
Domain 5. Exposure er	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 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 test animals were 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 and five animals were used in each repli- cate.			
Continued on next page							

Dibutyl Phthalate

		cor	ntinued from previous	page			
Study Citation:	Jonsson, S., I and Chemistr	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	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:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Larvae					
Health Outcome:	Immobilizati	on					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	789536						
Domain	Metric Rating Comments						
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently 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.			
	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 on outcomes unrelated to the exposure.			
Domain 7: Data Presenta	ation and Analy	ysis					
	Metric 21:	Statistical Methods	High	EC10 and EC50 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 EC10 and EC50 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 EC10 and EC50 values were reported.

Overall Quality Determination

Uninformative

Study Citation:	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.							
Duration: Exposure Route,	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) Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile						
Media, Path: Taxa, Species, Age:	Invertebrate:							
Health Outcome:	Mortality	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	1336024	336024						
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	Chemical purity was reported as 99.5 %.				
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	No mortalities were 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	No details were provided.				
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured after Day 1 and reported in Table 2.				
	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 suitable for a dose				
		Spacing of Exposure Levels	0	response.				
	Metric 12:	Testing at or Below Solubility Limit	Medium	Most exposure concentrations were below the water solubility limit. The high concentra- tion was close.				
Domain 4. Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. "Test animals for both the acute immobilization tests (range finding test) and the repro- duction test were collected when the animals were less than 24 h old."				
	Metric 14:	Acclimatization and Pretreatment	Medium	Pretreatment conditions were the same for control and exposed organisms, but details were limited				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were five daphnids per treatment with two replicates.				
		,						
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions were not sufficiently reported to evaluate if adequate.				
		Conti	nued on next pa	ge				

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Environmental Hazard Evaluation

HERO ID: 1336024 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation:	Mccarthy, J. Toxicology a	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.					
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:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1336024						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was reported in the text.			
	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	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 in text form.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	Range finding test: "In the acute mortality test (range-finding test), all D. magna were dead after 48 h of exposure to nominal concentrations of 7.5 and 10.0 mg/L DBP. At the lower doses of 3.0, 1.0 and 0.5 mg/L DBP and in controls, all animals survived, except for one individual at 3.0 mg/L. The LC50 (lethal concentration to 50% of the test population) is between 3.0 and 7.5 mg/L DBP. Although a probit analysis cannot be performed, because this procedure requires two responses that are between 0 and 100% mortality, a nonparametric analysis was developed for steep dose-response bioassays (Schmoyer, Beauchamp and McCarthy, manuscript in preparation). The LC50 was estimated using this method and was equal to 5.2 mg/L, with 95% confidence limits of 4.7 and 5.6 mg/L."						

Overall Quality Determination

Medium

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	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)					
Taxa, Species, Age:	Invertebrate;	nvertebrate; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	5043468						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT: 1				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#			
	Metric 2: Metric 3:	Test Substance Purity	Low High	The test substance identity was not analytically verified by the performing laboratory. Chemical purity reported as $>90\%$			
	Wietrie 5.	Test Substance Fullty	Ingn	Chemical purity 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 groups was reported			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups			
Domain 3: Exposure Ch	aracterization	Environmental Sectors / Track Madia	T				
	Metric 7:	Preparation	Low	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 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		T7' 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 organisms and replicates were suitable			
		Replicates per Group					
Domain 5: Outcome As	sessment		т				
	Metric 16:	Adequacy of Test Conditions	LOW High	Details of environmental conditions of test system were not reported The outcome assessment methodology reported the intended outcome			
	weute 17:	Outcome Assessment Methodology	підіі	The outcome assessment methodology reported the intended outcome			
Continued on next page							

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Dibutyl Phthalate

	continued from previous 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				
	Environment	654:969-977.				
Duration:	Overall Dura	ition: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-96	h)		
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:	Mechanistic	Biomarkers (exposure and effect)				
Chemical:	Dibutyl phth	alate (DBP)				
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 Coi	ntrol	· · · ·			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups		
	M-4	Design and Procedures	II: -h			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data Present	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 22.	Explanation of Unexpected Outcomes	High	There were no unevroated outcome		
	Metric 25.	Explanation of Onexpected Outcomes	Ingn	There were no unexpected outcome		
Additional Comments:	s: 24 hr exposure, measured at 96 hr					
Overall Qualit	ty Detern	nination	Medium			

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

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 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)					
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Mortality Dibutyl phth	Arthropods; <i>Daphnia magna</i> ; Juvenile alate (DBP)				
HERO ID:	5043468					
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 >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	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	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 (48 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 Organis	m					
Domain 1. 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		
	Metric 15:	Conditions Number of Organisms and	Medium	The number of exposure groups and spacing of exposure levels were suitable		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Details of environmental conditions of 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-		
		A55555111C111		100		

Domain 6: Confounding / Variable Control

Dibutyl Phthalate

... continued from previous page **Study Citation:** 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. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) 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; Daphnia magna; Juvenile Health Outcome: Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 5043468 Domain Metric Rating Comments Confounding Variables in Test Metric 19: 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 Presentation and Analysis Metric 21: Statistical Methods Uninformative Statistical analysis was not conducted. Metric 22: Uninformative Reporting of Data Data presentation was inadequate Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcome Additional Comments: None **Overall Quality Determination** Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

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.	Overall Dur Aquatic (fre	Werall Duration: > 21 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)						
Media. Path:	require (neshwater), water, we determined by study additis (ne., chemical of interest in exposure water, but anable to determine exact uptake for							
Taxa, Species, Age:	Invertebrate	: Arthropods: Daphnia magna: Iuvenile						
Health Outcome	Reproductiv	e/Teratogenic						
Chemical.	Dibutyl phth	palate (DBP)						
HERO ID:	5043468							
Domain	0010100	Metric	Rating	Comments				
Domain 1: Test Substar	nce		8					
	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	Matric 1.	Negative Controls	High	Study authors reported using an appropriate concurrent pagative control group				
	Metric 4.	Negative Control Despense	High	Study authors reported using an appropriate concurrent negative control group.				
	Metric 3:	Dandaminad Allasatian	High L	The biological response of the negative control groups was reported.				
	Metric 6:	Randomized Allocation	LOW	Researchers did not report now organisms were allocated to study groups.				
Domain 3: Exposure C	haracterization							
	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 are unlikely to have a substantial impact on results.				
		Administration	Ŧ					
	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/	Low	Only two exposure groups ware reported and appropriate for the study type.				
	Wieure 11.	Spacing of Exposure Levels	LOW	Only two exposure groups were reported.				
	Metric 12:	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	Medium	The number of organisms and replicates were suitable				
	meule 13.	Replicates per Group	wiedium	The number of organisms and represess were suitable.				
		Replicates per Group						
Domain 5: Outcome As	ssessment							
	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.				
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-				
		Assessment		ited.				

Dibutyl Phthalate

Study Citation:	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in Daphnia magna. Science of the Total						
Duration	Environment 654:969-977. Overall Duration: > 21 days: Exposure Duration: 0 - 4 days (0-96b)						
Euroguna Douto	Δ quatic (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 (rreshwater); 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:	Dibutyl phthalate (DBP)						
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	veis					
Domain 7. Data Present	Matric 21.	Statistical Methods	Low	Statistical analysis was performed but not described adequately			
	Metric 21.	Statistical Methods	LOW	Statistical analysis was performed but not described adequatery.			
	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 form is	evaluating the progeny results.					
Overall Qualit	ty Detern	nination	Medium				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

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 Dur	erall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	e, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact up							
Media, Path:								
Taxa, Species, Age:	Invertebrate	; Arthropods; Daphnia magna; Juvenile						
Health Outcome:	Nutritional a	& Metabolic						
Chemical:	Dibutyl phth	nalate (DBP)						
HERO ID:	5043468							
Domain		Metric	Rating	Comments				
Domain 1: Test Substa	nce							
	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 >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 groups was reported				
	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	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 are 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 10.	Number of Exposure Groups/	Low	Only two exposure groups ware reported and appropriate for the study type				
	Metric 11:	Spacing of Exposure Lough	Low	Only two exposure groups were reported				
	Metric 12.	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate				
	Wette 12.	Testing at of Below Solubility Linit	Ingi	The solvent concentration was appropriate				
Domain 4: Test Organi	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	Medium	The number of organisms and replicates were suitable				
	Wette 15.	Replicates per Group	Wiedrum	The number of organisms and replicates were suitable				
Domain 5: Outcome A	ssessment							
	Metric 16:	Adequacy of Test Conditions	Low	Details of environmental conditions of 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				

Dibutyl Phthalate

HERO ID: 5043468 Table: 4 of 5

	a b		1 1 .				
Study Citation:	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on 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)						
Evnosura Routa	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Madia Dath	Aqualic (ireshwater), water, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Paul:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Nutritional d	x Metabolic					
Chemical:	Dibutyl phth	alate (DBP)					
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			
Damain 7. Data Data ant							
Domain /: Data Present	Metric 21:	ysis Statistical Mathods	Low	Statistical analysis was performed but not described adoquately.			
	Matria 22:	Benerting of Data	Low	Dete for evenewre related for dings were presented for each treatment and control group			
	Metric 22:	Exploration of University of Outpower	Пigli II:-h	Data for exposure-related findings were presented for each treatment and control group			
	Metric 25:	Explanation of Unexpected Outcomes	High	I here were no unexpected outcome			
Additional Comments:	lipid accumu	ilation					
Overall Qualit	ty Detern	nination	Medium				

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 Dur	t 654:969-977. ation: 0 - 4 days (0-96h): Exposure Duration:	0 - 4 days (0-96h)	
Exposure Route,	Aquatic (fre	shwater); Water; Not determined by study aut	hors (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; Embryo		
Health Outcome:	Mortality			
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	5043468			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	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	Hıgh	Chemical purity 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 groups 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 C	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare
	1.100110 /1	Preparation	2011	test concentrations
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are 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 (96 hr) was reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/	Low	Only two exposure groups were reported
	Weute II.	Spacing of Exposure Levels	Low	Only two exposure groups were reported
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate
Domain 4: Test Organi	em			
Domain 4. Test Olgani	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		an procession conditions were are 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 A	ssessment			
	Metric 16:	Adequacy of Test Conditions	Low	Details of environmental conditions of 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

Dibutyl Phthalate

... continued from previous page **Study Citation:** 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. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) 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; Daphnia magna; Embryo Health Outcome: Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 5043468 Domain Metric Rating Comments Confounding Variables in Test Metric 19: 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 Presentation and Analysis Metric 21: Statistical Methods Uninformative Statistical analysis was not conducted. Metric 22: Uninformative Reporting of Data Data presentation was inadequate Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcome Additional Comments: with ephippia **Overall Quality Determination** Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Shen, C., We PeerJ 7(3):e6 Overall Dura Aquatic (fres	Shen, C., Wei, J., Wang, T., Wang, Y. (2019). Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. PeerJ 7(3):e6584. 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; Mechanistic- Dibutyl phth	Arthropods; <i>Daphnia magna</i> ; Adult Oxidative stress (including redox biology)					
HERO ID:	5433053	diale (DDI)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.			
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Sinopharm Chemical Reagent Co. Ltd, and verified by GC-MS.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control without DBP and a solvent control.			
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Figures 1-6 and were appropriate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the Daphnids were allocated into study groups.			
Domain 3 [,] Exposure Ch	aracterization						
2 chian et 24posare ett	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test substance. The test system was a static system.			
	Metric 8:	Consistency of Exposure Administration	High	All tests were for 24 or 48h in 100mL of test solution with a 12L:12D photoperiod at 22C. Tests were conducted in dechlorinated water.			
	Metric 9:	Measurement of Test Substance	High	Test concentrations were measured using GC-MS.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 48h with assessments conducted at 24 and 48h.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only two exposure levels reported, which is lower than is typical. Spacing was adequate to observe a response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent and had a solvent control with an appropriate amount of solvent.			
Domain 4: Test Organist	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Key Laboratory of Hydrobiology at Dalian Ocean University. The age of the Daphnia was appropriate for the study.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the Daphnia were acclimated to test conditions.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 10 Daphnia per test chamber, and each concentration was tested with five replicates.			

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Environmental Hazard Evaluation

HERO ID: 5433053 Table: 1 of 4

		conti	nued from p	previous page		
Study Citation:	Shen, C., We PeerJ 7(3):e	ei, J., Wang, T., Wang, Y. (2019). Acute tox 6584.	icity and resp	ponses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna.		
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	on: 0 - 4 days	s (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; <i>Daphnia magna</i> ; Adult					
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5433053					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	The tests were conducted with dechlorinated water at 22C with a 12L:12D photoperiod. Prior to the study, the Daphnids were fed Scenedesmus sp. They were not fed for the duration of the test.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–changes in H2O2 concentrations, lipid peroxidation, T-AOC, SOD, CAT, and GST.		
	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	τ			
	Metric 19:	Design and Procedures	LOW	I ne study did not provide enough information to allow a comparison of environmental conditions. It was not 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 Present	ation and Anal	veis				
2 onium 7. Dutu i reselle	Metric 21	Statistical Methods	High	Statistical methods included two-way ANOVA with Duncan's multiple range test		
	Metric 22:	Reporting of Data	High	The biological control response and the exposure response were reported in Figures 1-6 and were appropriate 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 of the evaluation was on the effect of DBP on H2O2, lipid peroxidation, T-AOC, SOD, CAT, and GST in D. magna adults. The mechanistic outcome of oxidative stress was selected as the outcome of interest. The supplemental data was not included in the Distiller download, but was provided via a link in the paper.					
Overall Qualit	ty Deterr	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	ponses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. (0-96h) chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Mechanistic-	-Oxidative stress (including redox biology)		
HERO ID:	5433053	alate (DBP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Sinopharm Chemical Reagent Co. Ltd, and verified by GC-MS.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control without DBP and a solvent control.
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Figures 1-6 and were appropriate for the outcomes of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the Daphnids were allocated into study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test substance. The test system was a static system.
	Metric 8:	Consistency of Exposure Administration	High	All tests were for 24 or 48h in 100mL of test solution with a 12L:12D photoperiod at 22C. Tests were conducted in dechlorinated water.
	Metric 9:	Measurement of Test Substance	High	Test concentrations were measured using GC-MS.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 48h with assessments conducted at 24 and 48h.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure levels reported, which is lower than is typical. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent and had a solvent control with an appropriate amount of solvent.
Domain 4: Test Organisi	n			
-	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Key Laboratory of Hydrobiology at Dalian Ocean University. The age of the Daphnia was appropriate for the study.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the Daphnia were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 Daphnia per test chamber, and each concentrations was tested with 5 replicates.
Domain 5: Outcome Ass	sessment			

Environmental Hazard Evaluation

HERO ID: 5433053 Table: 2 of 4

		conti	nuea from p	orevious page		
Study Citation:	Shen, C., We PeerJ 7(3):e	ei, J., Wang, T., Wang, Y. (2019). Acute tox 6584.	icity and resp	ponses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna.		
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:	Mechanistic-Oxidative stress (including redox biology)					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5433053					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	The tests were conducted with dechlorinated water at 22C with a 12L:12D photoperiod. Prior to the study, the Daphnids were fed Scenedesmus sp. They were not fed for the duration of the test.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–changes in H2O2 concentrations, lipid peroxidation, T-AOC, SOD, CAT, and GST.		
	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 o. Comoundang	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	11104110 191	Design and Procedures	2011	conditions—it was not 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 Present	ation and Anal	lycic				
2 cinain 7. Data i reselle	Metric 21	Statistical Methods	High	Statistical methods included two-way ANOVA with Duncan's multiple range test		
	Metric 22:	Reporting of Data	High	The biological control response and the exposure response were reported in Figures 1-6 and were appropriate 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 of the evaluation was on the effect of DBP on H2O2, lipid peroxidation, T-AOC, SOD, CAT, and GST in D. magna neonates. The mechanistic outcome of oxidative stress was selected as the outcome of interest. The supplemental data was not included in the Distiller download, but was provided via a link in the paper.					
Overall Qualit	a link in the	paper. nination	High			

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Study Citation:	Shen, C., Wei, J., Wang, T., Wang, Y. (2019). Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. PeerJ 7(3):e6584.					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (free	ation: 0 - 4 days (0-96h); Exposure Durations of the study shwater); Water; Not determined by study	on: 0 - 4 days authors (i.e.,	(0-96h) chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Adult				
Health Outcome: Chemical:	Mortality Dibutyl phth	alate (DBP)				
HERO ID:	5433053					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Sinopharm Chemical Reagent Co. Ltd, and verified by GC-MS.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control without DBP and a solvent control.		
	Metric 5:	Negative Control Response	High	The negative control response and the solvent control response were both reported in the supplementary data and were adequate.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the Daphnids were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test substance. The test system was a static system.		
	Metric 8:	Consistency of Exposure Administration	High	All tests were for 24 or 48h in 100mL of test solution with a 12L:12D photoperiod at 22C. Tests were conducted in dechlorinated water.		
	Metric 9:	Measurement of Test Substance	High	Test concentrations were measured using GC-MS.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 48h with LC50 values calculated at 24 and 48h.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 5 exposure levels reported, but the highest concentrations did not have com- plete mortality.		
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent and had a solvent control with an appropriate amount of solvent. The solvent control response was adequate for the outcome of interest.		
Domain 4: Test Organis	n					
	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Key Laboratory of Hydrobiology at Dalian Ocean University. The age of the Daphnia was appropriate for the study.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the Daphnia were acclimated to test conditions.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 Daphnia per test chamber, and each concentrations was tested with 5		
		Replicates per Group		replicates.		

Domain 5: Outcome Assessment

Environmental Hazard Evaluation

		contin	nued from p	revious page			
Study Citation:	Shen, C., We PeerJ 7(3):e6	Shen, C., Wei, J., Wang, T., Wang, Y. (2019). Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. PeerJ 7(3):e6584.					
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duratio	n: 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; Adult						
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5433053						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	The tests were conducted with dechlorinated water at 22C with a 12L:12D photoperiod. Prior to the study, the Daphnids were fed Scenedesmus sp. They were not fed for the duration of the test.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-LC50 values were reported.			
	Metric 18:	Consistency of Outcome	Low	Little detail was provided on the outcome assessment protocol for the acute toxicity.			
		Assessment					
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		conditions-it was not 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 Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	LC50 values and their 95% confidence issues were determined using Probit.			
	Metric 22:	Reporting of Data	High	The biological control response and the exposure response were reported in the supple- mentary data. LC50 values were reported in Table 1.			
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. 95% confidence intervals were reported in Table 1.			
Additional Comments:	This portion were reporte	of the evaluation was on the acute toxicity of the supplemental data was not included	of DBP on D in the Distill	n magna adults. Mortality was selected as the outcome of interest because LC50 values er download, but was provided via a link in the paper.			

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Shen, C., Wei, J., Wang, T., Wang, Y. (2019). Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. Peerl 7(3):e6584					
Duration: Exposure Route, Media, Path:	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				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5433053					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.		
	Metric 2:	lest Substance Source	High	The source of the DBP was reported to be Sinopharm Chemical Reagent Co. Ltd, and verified by GC-MS.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
e	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control without DBP and a solvent control.		
	Metric 5:	Negative Control Response	High	The negative control response and the solvent control response were both reported in the supplementary data and were adequate.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the Daphnids were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
ľ	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test substance. The test system was a static system.		
	Metric 8:	Consistency of Exposure Administration	High	All tests were for 24 or 48h in 100mL of test solution with a 12L:12D photoperiod at 22C. Tests were conducted in dechlorinated water.		
	Metric 9:	Measurement of Test Substance	High	Test concentrations were measured using GC-MS.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 48h with LC50 values calculated at 24 and 48h.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 5 exposure levels reported, but the highest concentrations did not have com- plete mortality.		
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent and had a solvent control with an appropriate amount of solvent. The solvent control response was adequate for the outcome of interest.		
Domain 4. Test Organisi	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Key Laboratory of Hydrobiology at Dalian Ocean University. The age of the Daphnia was appropriate for the study.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the Daphnia were acclimated to test conditions.		
	Metric 15:	Conditions Number of Organisms and	Medium	There were 10 Daphnia per test chamber, and each concentrations was tested with 5		
		Replicates per Group		replicates.		

Domain 5: Outcome Assessment

Environmental Hazard Evaluation

		conti	nued from p	revious page					
Study Citation:	Shen, C., We PeerJ 7(3):ee	Shen, C., Wei, J., Wang, T., Wang, Y. (2019). Acute toxicity and responses of antioxidant systems to dibutyl phthalate in neonate and adult Daphnia magna. PeerJ 7(3):e6584.							
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								
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	5433053								
Domain		Metric	Rating	Comments					
	Metric 16:	Adequacy of Test Conditions	High	The tests were conducted with dechlorinated water at 22C with a 12L:12D photoperiod. Prior to the study, the Daphnids were fed Scenedesmus sp. They were not fed for the duration of the test.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-LC50 values were reported.					
	Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the outcome assessment protocol for the acute toxicity.					
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 conditionsit was not 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 Present	ation and Anal	ysis							
	Metric 21:	Statistical Methods	High	LC50 values and their 95% confidence issues were determined using Probit.					
	Metric 22:	Reporting of Data	High	The biological control response and the exposure response were reported in the supple- mentary data. LC50 values were reported in Table 1.					
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. 95% confidence intervals were reported in Table 1.					
Additional Comments:	This portion values were	of the evaluation was on the acute toxicity reported. The supplemental data was not in	y of DBP on cluded in the	D. magna neonates. Mortality was selected as the outcome of interest because LC50 Distiller download, but was provided via a link in the paper.					

Overall Quality Determination

High

Study Citation: Duration:	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221. 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 rou						
Media, Path:	T (1)						
Taxa, Species, Age: Health Outcome:	Invertebrate;	Arthropods; Daphnia magna; Juvenile					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	4829279						
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	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.			
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.			
Domain 2: Test Design							
e	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.			
	Metric 5:	Negative Control Response	High	Survival in experimental controls and vehicle controls was 100%.			
	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. Exposure was conducted in 100mL glass beakers.			
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not 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	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	High	The solvent concentration was appropriate.			
Domain 1: Test Organia							
Domain 4. Test Organisi	Metric 13.	Test Organism Characteristics	Medium	The test organisms were adequately described, but culture origin was not reported			
	Metric 14	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms			
	1,10010 1 4 .	Conditions	mgn	in pretention 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 charac- terize toxicological effects (10 organisms per treatment per beaker and replicated five times).			
Domain 5: Outcome Ass	sessment						

HERO ID: 4829279 Table: 1 of 1

		contin	nued from p	revious page			
Study Citation:	Wei, J., Sher magna. Bull	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.					
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						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	4829279						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported.			
	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	Low	The study did not provide enough information to allow a comparison of environmental			
	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	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text and tables.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industrial Health 18(5):225-235.							
Duration: Exposure Route,	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:	Inventabuata	Arthropoda, Danhuig waang Adult						
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1332818							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	ce		Ŧ					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be ChemSevice in West Chester, PA, USA, but it was not reported if it was analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. Acetone was used in the control solvent.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.				
	Metric 6:	Randomized Allocation	Medium	It was reported that the D. magna were randomly assigned to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	It was not reported how the test media was prepared. Test system was static renewal				
		Preparation		with media being refreshed every 3 days. No information was provided on the type of experimental vessel (glass or plastic) used.				
	Metric 8:	Consistency of Exposure	Low	Exposures were administered for 6 days in 30mL of test solution with one adult organ- isms per test chamber. There were 40 replicates for each treatment				
	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:	Concentration Exposure Duration and Frequency	Medium	The study duration was reported to be for 6 days. This is longer than is typical for acute studies, and much shorter than chronic studies.				
	Metric 11:	Number of Exposure Groups/	Low	A range of test concentrations used (10-100ug/L) for DBP was provided, but actual test concentrations were not reported				
	Metric 12:	Testing at or Below Solubility Limit	High	An appropriate solvent and a solvent control were used in this study.				
Domain 4: Test Organis	n		Ŧ					
	Metric 13:	Test Organism Characteristics	Low	The study reported that adult D. magna were used in the study. Adult D. magna would not be as sensitive as neonates. This could affect the outcomes of the study.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated before the study.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There was one organism per test chamber and 40 replicates per treatment.				

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Environmental Hazard Evaluation

HERO ID: 1332818 Table: 1 of 3

		conti	nued from p	previous page			
Study Citation: Duration: Exposure Route,	 Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industrial Health 18(5):225-235. 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>Daphnia magna</i>; Adult Development/Growth Dibutyl phthalate (DBP) 1332818 						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:							
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept at 21C with a 6L:18D photoperiod. This photoperiod is not typ- ical of parthenogenic reproduction, though authors reported they normally see about 25% male production. It was reported they were fed, but the feeding regimen was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-male production and decreased growth.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment was lim- ited and in cases not reported.			
Domain 6: Confounding	r / 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	The G-test and the Student's T-test were used for statistical analysis depending on which outcome was being analyzed.			
	Metric 22:	Reporting of Data	Low	Data for each treatment level and control response were not reported. Only some of the data was reported.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	This portion adults expos	of the evaluation was on the decreased group of the DBP.	owth in term	s of deformities and adult size, as well as the increased number of males in D. magna			
Overall Quali	ty Deterr	nination	Low				

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HERO ID: 1332818 Table: 2 of 3

Study Citation: Duration: Exposure Route, Media, Path:	Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industrial Health 18(5):225-235. 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; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1332818					
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 DBP was reported to be ChemSevice in West Chester, PA, USA, but it was not reported if it was analytically verified.		
	Metric 3:	Test Substance Purity	Low	The purity was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. Acetone was used in the control solvent.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.		
	Metric 6:	Randomized Allocation	Medium	It was reported that the D. magna were randomly assigned to study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5: Exposure Cri	Metric 7:	Experimental System/Test Media Preparation	Low	It was not reported how the test media was prepared. Test system was static renewal with media being refreshed every 3 days. No information was provided on the type of experimental vessel (glass or plastic) used.		
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered for 6 days in 30mL of test solution with one adult organ- isms per test chamber. There were 40 replicates for each treatment.		
	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:	Concentration Exposure Duration and Frequency	Low	The study duration was reported to be for 6 days. This is longer than is typical for acute studies, and much shorter than chronic studies.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations used (10-100ug/L) for DBP was provided, but actual test concentrations were not reported.		
	Metric 12:	Testing at or Below Solubility Limit	High	An appropriate solvent and a solvent control were used in this study.		
Domain 4: Test Organisi	m					
_	Metric 13:	Test Organism Characteristics	Low	The study reported that adult D. magna were used in the study. Adult D. magna would not be as sensitive as neonates. This could affect the outcomes of the study.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated before the study.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There was one organism per test chamber and 40 replicates per treatment.		
Domain 5: Outcome Ass	sessment	· · ·				

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Environmental Hazard Evaluation

HERO ID: 1332818 Table: 2 of 3

		conti	nued from p	revious page					
Study Citation:	Kashian, D. Health 18(5)	Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industrial Health 18(5):225-235.							
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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; Adult								
Health Outcome:	Mortality								
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	1332818								
Domain		Metric	Rating	Comments					
	Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept at 21C with a 6L:18D photoperiod. This photoperiod is not typ- ical of parthenogenic reproduction, though authors reported they normally see about 25% male production. It was reported they were fed, but the feeding regimen was not reported.					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-survivorship.					
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment was lim- ited and in cases not reported.					
Domain 6: Confoundin	or / Variable Co	ntrol							
Domain 0. Comountum	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental					
	Wieure 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 Presen	tation and Anal	vsis							
	Metric 21:	Statistical Methods	High	The G-test and the Student's T-test were used for statistical analysis depending on which outcome was being analyzed.					
	Metric 22:	Reporting of Data	Low	Data for each treatment level and control response were not reported. Only some of the data was reported.					
	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 decreased survivorship in D. magna after exposure to DBP. Mortality was selected as the outcome of interest.

Overall Quality Determination

Low

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HERO ID: 1332818 Table: 3 of 3

Study Citation: Duration: Exposure Route, Media, Path:	Kashian, D. J Health 18(5) Overall Dura Aquatic (fres	Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industrial Health 18(5):225-235. 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; Adult					
Health Outcome:	Reproductive	e/Teratogenic					
HERO ID:	1332818						
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 DBP was reported to be ChemSevice in West Chester, PA, USA, but it was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity was not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. Acetone was used in the control solvent.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.			
	Metric 6:	Randomized Allocation	Medium	It was reported that the D. magna were randomly assigned to study groups.			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	It was not reported how the test media was prepared. Test system was static renewal with media being refreshed every three days. No information was provided on the type of experimental vessel (glass or plastic) used.			
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered for six days in 30mL of test solution with one adult organ- ism per test chamber. There were 40 replicates for each treatment.			
	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:	Concentration Exposure Duration and Frequency	Low	The study duration was reported to be for six days. This is longer than is typical for acute studies, and much shorter than chronic studies.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations used (10-100ug/L) for DBP was provided, but actual test concentrations were not reported.			
	Metric 12:	Testing at or Below Solubility Limit	High	An appropriate solvent and a solvent control were used in this study.			
Domain 4. Test Organic							
20man 1. rost organis.	Metric 13:	Test Organism Characteristics	Low	The study reported that adult D. magna were used in the study. Adult D. magna would not be as sensitive as neonates. This could affect the outcomes of the study.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated before the study.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There was one organism per test chamber and 40 replicates per treatment.			
Domain 5: Outcome Ass	sessment	· · ·					

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Environmental Hazard Evaluation

HERO ID: 1332818 Table: 3 of 3

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Study Citation:	Kashian, D. R., Dodson, S. I. (2002). Effects of common-use pesticides on developmental and reproductive processes in Daphnia. Toxicology and Industria Health 18(5):225-235.							
Duration:	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:	Invertebrate; Arthropods; Daphnia magna; Adult							
Health Outcome:	Reproductiv	e/Teratogenic						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1332818							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Organisms were kept at 21C with a 6L:18D photoperiod. This photoperiod is not typ- ical of parthenogenic reproduction, though authors reported they normally see about 25% male production. It was reported they were fed, but the feeding regimen was not reported.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-fecundity.				
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-				
		Assessment		ited and in some cases not reported.				
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 Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	The G-test and the Student's T-test were used for statistical analysis depending on which outcome was being analyzed.				
	Metric 22:	Reporting of Data	Low	Data for each treatment level and control response were not reported. Only some of the data was reported.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				

Overall Quality Determination

Low

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: 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:	Invertebrate;	Arthropods; Daphnia magna; Adult					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
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	Medium	One test substance (DBoP, di-n-butyl ortho) was commercially purchased and of high purity (>99%). Chemical was also synthesized for tests and purity not analytically verified. Iso and tere forms also synthesized.			
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	Organisms were randomly distributed among treatments and randomly subsampled for measurements.			
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. DBP 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.			
	Metric 10.	Concentration Exposure Duration and Frequency	Hioh	Exposure was appropriate and followed standard ASTM protocols			
	Metric 11:	Number of Exposure Groups/	High	Range of concentrations allowed for calculation of reproduction.			
	Matria 12:	Spacing of Exposure Levels	Madium	Some test concentrations were at an show calculation to reach weif estimated and an entry			
	Metric 12:	Testing at or Below Solubility Limit	Medium	tions was provided and care taken to ensure minimal degradation or loss of test sub- stance during experiments.			
Domain 4: Test Organisi	n						
C	Metric 13:	Test Organism Characteristics	High	Test organism was obtained from a reliable source and test organism details were pro- vided.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Minimal details on pretreatment were provided.			
Continued on next page							

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HERO ID: 5774391 Table: 1 of 2

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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: 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:	• ·	· · · ·					
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Adult					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
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.			
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 (reproduction) 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	tation 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. Author provided dis- cussion on different results measured in other papers.			
Additional Comments:	21d chronic	21d chronic exposure of Daphnia to DBP had a significant effect on reproduction.					

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.
	Environmental Toxicology and Chemistry 9(5):623-636.
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; Adult
Health Outcome:	Mortality
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	5774391

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
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	Medium	One test substance (DBoP, di-n-butyl ortho) was commercially purchased and of high purity (>99%). The chemical was also synthesized for tests, but the purity was not analytically verified. Iso and tere forms were also synthesized.
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	Organisms were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characteriz	ation		
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. DBP 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 9: Measurement of Test Substance	High	Exposure concentrations were measured using appropriate methods.
Metric	10: Exposure Duration and Frequency	High	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	Spacing of Exposure Levels 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 taken to ensure minimal degradation or loss of test sub- stance during experiments.
Domain 4: Test Organism			
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	Conditions 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms used follows standard ASTM protocol.
	Con	tinued on nex	t page

Environmental Hazard Evaluation

HERO ID: 5774391 Table: 2 of 2

		conti	nued from p	revious page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Defoe, D. L. Environment Overall Dura Aquatic (fres Invertebrate; Mortality Dibutyl phth 5774391	, Holcombe, G. W., Hammermeister, D. E., tal Toxicology and Chemistry 9(5):623-636 ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study a Arthropods; <i>Daphnia magna</i> ; Adult alate (DBP)	, Biesinger, k 5. - 21 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)
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	Study conditions (physiochemical characteristics, feeding details) are well documented. The outcome of interest (LC50) was appropriate. Outcomes were assessed consistently across groups.
Domain 6: Confounding	g / Variable Con Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High Medium	There were no variations or inconsistencies reported across study groups and environ- mental conditions are provided. There is no information to suggest differences among groups.
Domain 7: Data Present	tation and Anal Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Statistical methods were appropriate. Results for all treatments and outcomes were reported. Authors reported no unexpected outcomes and reported variance. Author provided dis- cussion on different results measured in other papers.
Additional Comments:	21d chronic	exposure of Daphnia to DBP had a signific	ant effect on	survival, and effects were dependent on form.

Overall Quality Determination

High

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	Environmental Research 6(1):84-90. 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	ransformation)				
Chemical:	Dibutyl phthalate (DBP)					
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	Purity and/or grade of test substance were not reported.		
Domain 2: Test Design						
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 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		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	Low	Few details of exposure administration were reported, but exposures were administered		
		Administration		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.		
	14.1.10	Spacing of Exposure Levels	TT' 1			
	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 Replicates per Group	Low	The number of test replicates was not reported.		
		Repleates per Group				
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	Medium	The outcome assessment methodology was not clearly reported.		
			Continued on next page			

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Dibutyl Phthalate

HERO ID: 1334646 Table: 1 of 1

			continued from previous pa	age		
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,	Aquatic (free	shwater); Water; Not determined by study a	uthors (i.e., chemical of inte	rest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Daphnia magna; Juvenile				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Dibutyl phth	alate (DBP)				
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	y / Variable Co	ntrol				
2011111 01 20110011011	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	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 Quali	ty Detern	nination	Uninformative			

Study Citation: Duration: Exposure Route, Media Path:	Mccarthy, J. Toxicology a Overall Dura Aquatic (free	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179. 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:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1336024					
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 99.5 %		
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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes although the non-solvent control had poor survival and fewer young		
	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	Daily renewals but few details were provided		
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured but are considerably lower than nominal con- centrations		
	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 suitable 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 Organisi	m		TT' 1			
	Metric 13:	Test Organism Characteristics	High	"Test animals for both the acute immobilization tests (range finding test) and the repro- duction test were collected when the animals were less than 24 h old."		
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms, details were limited		
	Metric 15:	Number of Organisms and Replicates per Group	Low	40 daphnids per treatment but no replicates		

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 1336024 Table: 1 of 3

		contin	ued from previ	ous page		
Study Citation:	Mccarthy, J. Toxicology a	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179				
Duration:	Overall Duration: 11 - 21 days: Exposure Duration: 11 - 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:	Invertebrate;	Arthropods; Daphnia magna; Juvenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1336024					
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	/ Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically- significant morality in the negative control group that may impact results for reproduc- tive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna."Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."		
	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 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	Unexpectedly low survival in clean control		
Additional Comments:	This form is of negative c to number of	to account for mortality in chronic Daphnia control organisms in this study as well as num f young per adult, but with such variability ir	magna test with ber of young pe controls introd	n results found in Table 3. There were serious concerns regarding the survivability or adult. The study authors attempted to bypass the survivability issue relate results uces uncertainty. The solvent control should be used as an alternative.		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media. Path:	Mccarthy, J. Toxicology a Overall Dura Aquatic (fres	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179. 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 Dibutyl phth 1336024	Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic alate (DBP)				
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 99.5 %.		
Domain 2: Test Design						
2 onnani 21 Tese 2 osign	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes although the non-solvent control had poor survival and fewer young.		
	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 Administration	Medium	Daily renewals occurred, but few details were provided.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but are considerably lower than nominal con- centrations.		
	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 suitable 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 Organisi	m Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. "Test animals for both the acute immobilization tests (range finding test) and the repro-		
	Metric 14.	Acclimatization and Pretreatment	High	duction test were collected when the animals were less than 24 h old."		
		Conditions	111511	were limited.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 40 daphnids per treatment, but no replicates used.		
Domain 5: Outcome Ass	ecoment					
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.		
		Contin	nued on next pa	ge		

HERO ID: 1336024 Table: 2 of 3

		contin	ued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Mccarthy, J. Toxicology a Overall Dura Aquatic (free Invertebrate;	F., Whitmore, D. K. (1985). Chronic toxicit and Chemistry 4(2):167-179. ation: 11 - 21 days; Exposure Duration: 11 - shwater); Water; Not determined by study at Arthropods; <i>Daphnia magna</i> ; Juvenile	y of di-n-butyl a 21 days uthors (i.e., chen	nd di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental nical of interest in exposure water, but unable to determine exact uptake route)
Health Outcome: Chemical:	Dibutyl phth	e/ Ieratogenic alate (DBP)		
HERO ID:	1336024			
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 Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically- significant morality in the negative control group that may impact results for reproduc- tive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	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	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	There was unexpectedly low survival in the clean control.
Additional Comments:	This form is negative con results to nut	for D. manga reproductive effects - total b trol organisms in this study as well as num mber of young per adult, but with such varia	broods and days ber of young pe ability in control	to primiparous instar. There were serious concerns regarding the survivability of r adult. The study authors attempted to bypass the survivability issue by relating s it introduces uncertainty. The solvent control should be used as an alternative.

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1336024 Table: 3 of 3

Study Citation: Duration:	Mccarthy, J. Toxicology a Overall Dura	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179. Overall Duration: 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 1336024	Arthropods; <i>Daphnia magna</i> ; Juvenile tt/Growth lalate (DBP)				
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	Chemical purity was reported as 99.5 %.		
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	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes, although the non-solvent control had poor survival and fewer young.		
	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	Daily renewals but few details were provided		
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured but are considerably lower than nominal con- centrations		
	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 suitable for a dose response		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit		
Domoin 4. Toot Orregie						
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. "Test animals for both the acute immobilization tests (range finding test) and the repro- duction test were collected when the animals were less than 24 h old."		
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms, details were limited		
	Metric 15:	Number of Organisms and Replicates per Group	Low	40 daphnids per treatment but no replicates		
Domain 5: Outcome Ag	sessment					
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health		
		Continued on next page				

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Environmental Hazard Evaluation

		contin	ued from previ	ous page			
Study Citation:	Mccarthy, J. Toxicology a	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Aquetic (freeshweter): Weter: Not determined by study outbors (i.e., chemical of interact in exposure water, but unable to determine event untake route)					
Exposure Route, Media Path.	Aquatic (free	snwater); water; Not determined by study at	itnors (i.e., chen	fical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate:	Invertebrate: Arthropods: Daphnia magna: Iuvenile					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1336024						
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 Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically- significant morality in the negative control group that may impact results for reproduc- tive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."			
	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	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	Unexpectedly low survival in clean control			
Additional Comments:	This form is organisms ir young per ac	to account for development of the Daphnic this study as well as number of young per dult, but with such variability in controls intr	ls found in Tabl adult. The stud oduces uncertai	e 4. There were serious concerns regarding the survivability of negative control dy authors attempted to bypass the survivability issue relate results to number of nty. The solvent control should be used as an alternative.			

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Modia, Path:	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 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:	Mortality	n ninopous, Dupinna magna, su tenne							
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	680120								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	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 >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 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 Prenaration	Medium	The experimental system and methods for preparation of 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 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 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								
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	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								
Domain 5. Outcome Ass	Metric 16.	A dequacy of Test Conditions	High	Organism environmental conditions were conducive to maintenance of organism health					
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome					
		Cont	inued on nex	t page					

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HERO ID: 680120 Table: 1 of 2

		contin	nued from p	previous page			
Study Citation:	Rhodes, J. E	E., Adams, W. J., Biddinger, G. R., Robilla	ırd, K. A., C	Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and			
Duration	rainbow trou	raindow from (Oncornynchus mykiss). Environmental foxicology and Chemistry 14(11):1907-1970.					
Duration: Evnosuro Douto	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route, Modia Dath.	Aquatic (free	sinwater), water, not determined by study a	utilors (1.e.,	chemical of interest in exposure water, but unable to determine exact uptake foute)			
Tava Spacias Agas	Income have a des Dan have a man an Income in						
Iaxa, Species, Age:	Mortality	Artinopous, Daprina magna, Juvenne					
Chamical	Dibutul abth	alata (DDD)					
	680120	alate (DBP)					
	080120						
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	e				
	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 Quali	Overall Quality Determination High						

Study Citation: Duration:	Rhodes, J. E rainbow trou Overall Dura	., Adams, W. J., Biddinger, G. R., Robill t (Oncorhynchus mykiss). Environmental tion: 11 - 21 days; Exposure Duration: 11	ard, K. A., C Toxicology a - 21 days	Borsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and nd Chemistry 14(11):1967-1976.	
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)	
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Dibutyl phthe 680120	Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic alate (DBP)			
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	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 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 2. Evenance Ch	anastanization				
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Media	Madium	The experimental system and methods for momentian of test modio years described in	
	Metric 7.	Preparation	Medium	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/ Spacing of Exposure Levels	High	The number of exposure groups and 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:	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 Ass	sessment		II: -h		
	Metric 16:	Adequacy of Test Conditions	High	organism nousing and environmental conditions were conducive to 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.	
	Continued on next page				

HERO ID: 680120 Table: 2 of 2

	continued from previous page						
Study Citation:	Rhodes, J. E rainbow trou	hodes, 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 inbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 14(11):1967-1976.					
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:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	680120						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / Variable Con Metric 19: Metric 20:	ntrol 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 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 Quali	Overall Quality Determination High						

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:	Environmen Overall Dura Aquatic (free	Environment 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;	Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
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%.			
Demain 2. T. (D.)							
Domain 2: Test Design	Matria 4.	Nagative Controls	Iliah				
	Metric 4: Matria 5:	Negative Control Response	High	Study authors reported using an appropriate concurrent negative control group.			
	Metric 5:	Randomized Allocation	Low	Pasearchers did not report how organisms were allocated to study groups			
	Wieure 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 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:	Concentration 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.			
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	mgn	The procounterne conditions were the sume for conditional exposed organisms.			
	Metric 15:	Number of Organisms and	Medium	The number of organisms and replicates were suitable.			
		Replicates per Group					
Domain 5: Outcome As	sassmant						
Domain 5. Outcome As	Metric 16.	A dequacy of Test Conditions	Low	Details of environmental conditions of the test system were not reported			
	Metric 17	Autome Assessment Methodology	LUW High	The outcome assessment methodology reported the intended outcome			
	wiente 17.	Outcome Assessment Methodology	Ingn	The outcome assessment methodology reported the intended outcome.			
Continued on next page							

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Dibutyl Phthalate

continued from previous 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				
	Environment 654:969-977.					
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:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
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	TT 1			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups.		
		Design and Procedures	TT' 1	771 11 00		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.		
Domain 7. Data Dresant	ation and Anal					
Domain 7. Data Fresent	Motrio 21:	ysis Statistical Mathada	Low	Statistical analysis was nonformed but not described adapted		
	Metric 21.	Benerting of Date	Low	Statistical analysis was performed but not described adequately.		
	Metric 22:	Explanation of Unaversated Outcomes	ПIgli Цісь	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:	length					
Overall Quality Determination Medium						

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Study Citation:	Wei, J., Shen	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia						
Duration:	Overall Dura	Werall Duration: 11 - 21 days; Exposure Duration: 11 - 21 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: Taya Species Age	Invertebrate	Arthropods: Daphnia magna: Adult						
Health Outcome:	Development	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	4829279							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	ce							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.				
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.				
Domain 2: Test Design								
U	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.				
	Metric 5:	Negative Control Response	High	Survival in experimental controls and vehicle controls was 100%.				
	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. The exposure was conducted with individual neonates in 20mL glass tubes.				
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not 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	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	High	The solvent concentration was appropriate.				
Domain 4: Test Organis	n Maria 12		N					
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but culture origin was not reported.				
	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 numbers of test organisms and replicates were reported and sufficient to character-				
		Replicates per Group		ize toxicological effects (15 organisms per treatment, in individual 20mL glass tubes).				

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 4829279 Table: 1 of 2

	continued from previous page						
Study Citation:	Wei, J., Shei magna, Bull	Vei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia nagna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.					
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;	Invertebrate; Arthropods; Daphnia magna; Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	4829279						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not 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.			
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	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	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text and tables.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	molting freq	uency, generational effects also reported					
Overall Quality Determination		High					

Study Citation: Duration: Exposure Route,	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221. 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 Dibutyl phthe 4829279	Arthropods; <i>Daphnia magna</i> ; Adult 2/Teratogenic alate (DBP)			
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	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.	
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >99%	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups	
	Metric 5:	Negative Control Response	High	survival in experimental controls and vehicle controls was 100%.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization	Encoder Contact (Contact Madia	T		
	Metric 7:	Preparation	Low	test concentrations. The exposure was conducted with individual neonates in 20mL glass tubes.	
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not 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	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	High	The solvent concentration was appropriate	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, culture origin 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 toricological circus (15 organishis per treathent, in mulvidual zonitz glass tudes).	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported	
Continued on next page					

HERO ID: 4829279 Table: 2 of 2

		contin	nued from p	previous page			
Study Citation:	Wei, J., She	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia					
Duration	magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.						
Fynosura Routa	Aquatic (fre	shwater): Water: Not determined by study a	uthors (i e	chemical of interest in exposure water, but upple to determine exact uptake 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: Arthropods: Danhnia magna: Adult						
Health Outcome	Reproductiv	e/Teratogenic					
Chamical.	Dibutyl phth	alate (DBP)					
HERO ID:	4829279						
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	y / 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			
	Medie 19.	Design and Procedures	Low	conditions			
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups			
Domain 7: Data Present	tation and Anal	vsis					
Domain 7. Data Presen	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 and tables			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	generational	effects also reported					
Overall Quali	ty Deterr	nination	High				

Study Citation: Duration: Exposure Route, Media. Path:	Bionomics,, Overall Dura Aquatic (free	Springborn (1984). Chronic toxicity of four tition: 11 - 21 days; Exposure Duration: > 2 shwater); Water; Not determined by study a	rteen phthalate es 21 days uthors (i.e., chen	sters to Daphnia magna with cover letter dated 032585. :95. nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Dibutyl phth 1316195	Arthropods; <i>Daphnia magna</i> ; Adult e/Teratogenic alate (DBP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only (DBP), 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		·		-
	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
	Metric 6:	Randomized Allocation	Medium	Organisms were allocated in an unbiased manner
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	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:	Consistency of Exposure Administration	High	Exposure was administered consistently across groups
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations were measured weekly, and there was significant decline in the concentrations of DBP over the course of the experiment.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate - 21 days
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	5 concentrations were tested. Actual concentrations were lower than nominal and de- clined over time despite the flow through design.
	Metric 12:	Testing at or Below Solubility Limit	Low	Some concentrations exceeded the water solubility limit. An Enhanced mixing tech- nique was used to deliver the chemical in the flow through system, but the loss of the test substance over time indicates that the chemical was instable in water.
Domain 4: Test Organis	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:	Conditions Number of Organisms and Replicates per Group	Low	There is no description of how many replicates there were per treatments 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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Dibutyl Phthalate

continued from previous page						
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 Reproductive/Teratogenic Dibutyl phthalate (DBP) 1316195					
Domain		Metric	Rating	Comments		
Domain 5: Outcome Ass	Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome	High High High	Environmental conditions were recorded and were consistent. Reproduction was assessed as cumulative number of offspring. Outcomes were assessed consistently across groups		
Domain 6: Confounding	/ Variable Cor	ntrol				
	Metric 19: Metric 20:	Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Medium	The water quality variables were comparable across treatments 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 Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Statistical tests were performed (not well described, authors cite an old method) but all data are presented in the tables for treatments and controls All data are presented in the tables for treatments and controls Outcomes were satisfactorily described		
Additional Comments:	None					
Overall Qualit	Overall Quality Determination Medium					

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:	Invertebrate; Mortality Dibutyl phth	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult Mortality Dibutyl phthalate (DBP)				
HERO ID:	1316195	316195				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only (DBP). No other information was given.		
	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.		
	Metric 6:	Randomized Allocation	Medium	Organisms were allocated in an unbiased manner.		
Domain 3: Exposure Ch	aracterization					
	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:	Consistency of Exposure	High	Exposure was administered consistently across groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Test concentrations were measured weekly, and there was significant decline in the concentrations of DBP over the course of the experiment.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration of 21 days was appropriate.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five concentrations were tested. Actual concentrations were lower than nominal and declined over time despite the flow through design.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Some concentrations exceeded the water solubility limit. An Enhanced mixing tech- nique was used to deliver the chemical in the flow through system, but the loss of the test substance over time indicates that the chemical was unstable in water.		
Domain 4: Test Organis	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 treatments 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 As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were recorded and were consistent.		
Continued on next page						

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Dibutyl Phthalate

		conti	inued from previo	us page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa Species Age:	Bionomics,, Overall Dura Aquatic (free	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)					
Health Outcome	Mortality	, Artinopods, <i>Dapnina magna</i> , Adun					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1316195	()					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed weekly, and reproduction was assessed as cumulative number of offspring.			
	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	The water quality variables were comparable across treatments.			
	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 tests were performed (not well described, authors cite an old method) but all data are presented in the tables for treatments and controls.			
	Metric 22:	Reporting of Data	High	All data are presented in the tables for treatments and controls.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily described.			
Additional Comments:	None						
Overall Qualit	Overall Quality Determination						

Study Citation:	Jr, Mayer, I	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	: 0 - 4 days (0-96h)				
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:	Invertebrate	; Arthropods; Gammarus pseudolimnaeus; N	ot Applicable (e.g., fungi	or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
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	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
C	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 2. Expedites Ch	anatanizatian						
Domain 5: Exposure Ch	Matria 7:	Experimental System/Test Media	Low	Concentrations of test substance ware not measured during the study			
	Weute 7.	Preparation	LOW	Concentrations of test substance were not measured during the study.			
	Metric 8:	Consistency of Exposure	Medium	Some details of exposure administration were reported and exposures were administered			
	Matria 0:	Administration Massurement of Test Substance	Low	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/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and 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						
Domain 1. 1050 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

Continued on next page ...

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Dibutyl Phthalate

		0	ontinued from previous p	age		
Study Citation:	Jr, Mayer, F Environmen	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	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; Gammarus pseudolimnaeus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	nalate (DBP)				
HERO ID:	1334646					
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 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	tation 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	Low	The study did not report any measures of variability.		
Additional Comments:	None					
Overall Quali	ty Deterr	nination	Uninformative			

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Study Citation:	tudy Citation: Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates.					
Derections	Environmen	tal Research 6(1):84-90.	21			
Duration: Evnosure Route	Aquatic (fre	ation: 11 - 21 days; Exposure Duration: 11 shwater): Water: Not determined by study a	- 21 days	terest in exposure water, but unable to determine exact untake route)		
Media. Path:	Aquatic (IIC	situate), water, not determined by study a	autors (i.e., chemical of m	terest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Gammarus pseudolimnaeus; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	ADME (bio	ADME (biotransformation)				
Chemical:	Dibutyl phth	nalate (DBP)				
HERO ID:	1334646					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce	Test Cubstance Identity	II: -h			
	Metric 1:	Test Substance Identity	Hign	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 Purity	LOW	Purity and/or grade of lest 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 2: Exposure Ch	araatarization					
Domain 5. Exposure en	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.		
	Metric 8:	Consistency of Exposure	Low	Few 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.		
	Metric 10.	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for		
	Methe 10.	Exposure Duration and Frequency	Ingn	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: Tast Organia	m					
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of the test enimels was not reported		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized		
	Metric 14.	Conditions	Low	The study did not report whether test organisms were accumulated.		
	Metric 15:	Number of Organisms and	Low	The number of test replicates was not reported.		
		Replicates per Group				
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 adequate		
	Metric 17.	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported		
	mult 17.	Sucome Assessment Methodology		The outcome assessment methodology was not clearly reported.		
Continued on next page						

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Dibutyl Phthalate

		con	tinued from previou	s page		
Study Citation:	Jr, Mayer, F Environment	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: 11 - 21 days; Exposure Duration: 11 - 21	l days			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study auth	ors (i.e., chemical of	nterest 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:	ADME (biot	ransformation)				
Chemical:	Dibutyl phthalate (DBP)					
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 Cor	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 Presenta	ation 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					

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:	Invertebrate; Behavioral	Arthropods; Gammarus pulex; Adult			
Chemical: HERO ID:	Dibutyl phth 732821	alate (DBP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Medium	Chemical only identified as DBP, a phthalate ester	
	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					
C	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 massures taken to enprepriately prepare	
	Metric 7.	Preparation	LOW	test concentrations, 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 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	Matri - 12	Test Organism Characteristics	Ma -1:		
	Matria 14:	A colimatization and Destroate and	Medium	I nere are minor reservations about the source of test organisms	
	wieuric 14:	Conditions	пıgn	An pretreatment conditions were the same for control and exposed organisms	
	Metric 15:	Number of Organisms and Paplicates per Group	Low	Replicates were not used or reported	
		Replicates per Gloup			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to 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	ge	

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HERO ID: 732821 Table: 1 of 3

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 Dura	ation: > 21 days; Exposure Duration: 4 - 10	days		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study at	thors (i.e., chem	ical 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:	Dibutyl phthalate (DBP)				
HERO ID:	732821				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome Assessment	Medium	Some details regarding the execution of the study protocol for outcome assessment were not reported	
Domain 6: Confounding	g / Variable Con Metric 19:	ntrol Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental	
	Wieute 19.	Design and Procedures	Low	condition	
	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	Highly variable results with no clear trends	
Additional Comments:	None				

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Study Citation: Duration: Exposure Route, Madia Bath	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 Dibutyl phth 732821	Arthropods; <i>Gammarus pulex</i> ; Adult alate (DBP)			
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Medium	The chemical was only identified as DBP, 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					
_ 511001 2. 1001 D 051511	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative 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	naracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. There is 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 Organis	m				
	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:	Number of Organisms and	Low	Replicates were not used or reported.	
		Replicates per Oloup			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to 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.	
		С	ontinued on next page .		

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 732821 Table: 2 of 3

		col	ntinued from previous	page		
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				
	Contaminati	on and Toxicology 46(1):159-166.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: 4 - 10 d	lays			
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study aut	hors (i.e., chemical of in	iterest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	; Arthropods; <i>Gammarus pulex</i> ; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	732821					
Domain		Metric	Rating	Comments		
Domain 6: Confoundin	g / Variable Co	ntrol	T			
	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.		
Domain 7: Data Presen	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

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HERO ID: 732821 Table: 3 of 3

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 Dura	ation: > 21 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:					
Taxa, Species, Age:	Invertebrate;	; Arthropods; Gammarus pulex; Adult			
Health Outcome:	ADME (biot	transformation)			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	732821				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce		-		
	Metric 1:	Test Substance Identity	Medium	Chemical only identified as DBP, a phthalate ester	
	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	Metric 4.	Negative Controls	High	Study authors reported using an appropriate concurrent negative control	
	Metric 5:	Negative Control Perponse	Low	The biological response of the pagetive control groups use not reported	
	Metric 5:	Pandomized Allocation	Low	Passarahars did not report how organisms were allocated to study groups	
	Wietric 0.	Kandonnized Anocation	LOW	Researchers did not report now organisms were anocated to study groups	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare	
	11101110 /1	Preparation	2011	test concentrations, 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 exposure was reported but steady state or time dependent results were not reported	
	Metric 11:	Number of Exposure Groups/	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	
			6		
Domain 4: Test Organisi	m				
	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 Replicates per Group	Low	Replicates were not used or reported	
Domain 5: Outcome Age	accmont				
Domain 5. Outcome Ass	Matria 16:	A deguage of Test Conditions	Uich	Organism anvironmental conditions wars conducius to maintenance of backtor -	
	Meute 10:	Aucquacy of rest Conditions	nigii	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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d Evaluation

		contin	ued from previ	ous page	
Study Citation:	Thurén, A.,	Woin, P. (1991). Effects of phthalate esters o	on the locomotor	activity of the freshwater amphipod Gammarus pulex. Bulletin of Environmental	
	Contaminati	on and Toxicology 46(1):159-166.			
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: 4 - 10	days		
Exposure Route,	Aquatic (free	shwater); Water; Not determined by study au	thors (i.e., chen	ical 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:	Dibutyl phthalate (DBP)				
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	
		Assessment		reponed	
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		condition	
	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 conducted, typical for BCF assessments	
	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	tv Detern	nination	Medium		

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Stade Citation	In Marrie I	Condens II O Walsh D E (1072) T		
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 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:	Invertebrate; Arthropods; Hexagenia bilineata; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	Dibutyl phth	nalate (DBP)		
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	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
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
	Mettre 0.	Randomized / mocarion	Low	Researchers and not report now organisms were anotated to study groups.
Domain 3: Exposure Ch	aracterization			
	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	Low	Few details of exposure administration were reported, but exposures were administered
		Administration		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
	Wieute 10.	Exposure Duration and Frequency	Ingn	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 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
	Weute 15.	Replicates per Group	Low	The number of test replicates was not reported.
		Replicates per Gloup		
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	Medium	The outcome assessment methodology was not clearly reported.
Continued on next page				
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Dibutyl Phthalate

		0	onunued from previous	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.								
	Environment	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	ransformation)							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)							
HERO ID:	1334646								
Domain		Metric	Rating	Comments					
	Metric 18:	Consistency of Outcome	Medium	Few details regarding the execution of the study protocol for the outcome assessment					
		Assessment		were 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					
	metho 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					
		1		attrition.					
Domain 7: Data Present	ation 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.					
		r ····································	-6	· · · · · · · · · · · · · · · · · · ·					
Additional Comments:	None								
Overall Qualit	v Detern	nination	Uninformative						

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Call, D. J., C	cox, D. A., Geiger, D. L., Genisot, K. I., M	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	Invironmental Toxicology and Chemistry	20(8):1805-18	315.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 -	10 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; Hyalella azteca; Not Applica	able (e.g., fun	gi or algae studies) or Not Reported				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	679311							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	Chemical was identified as a "single isomer" and the identify, including CASRN refer- enced in an outside paper, Call et al 2001.				
	Metric 2:	Test Substance Source	High	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 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	Biological response of control groups was appropriate as shown in Table 4.				
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	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 reported and consistent among different DBP treatments and controls.				
	Metric 9:	Measurement of Test Substance	High	Concentrations measured using HPLC as described in methods and cited reference (Call et al 2001)				
	Metric 10.	Exposure Duration and Frequency	High	Duration (10 day exposure) was appropriate for experimental design				
	Metric 11:	Number of Exposure Groups/	Medium	Exposure groups were acceptable and spanned 5 concentrations per test species in addi-				
	ineure II.	Spacing of Exposure Levels	mourum	tion to control; nominal doses unclear however measured doses reported in sediment and pore water.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via sediment.				
Domain 4. Test Organis	m							
2 chain 1. Test organis	Metric 13.	Test Organism Characteristics	Low	Source of test organism not reported				
	Metric 14	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to exposure not reported				
		Conditions	2011	recommendation of these of guillounds prior to exposure not reported.				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized 3 replicates of five different concentrations with 10 organisms per beaker and three sediment control replicates with 10 test organisms per beaker and three silica sand control replicates with 10 test organisms per beaker				

Environmental Hazard Evaluation

HERO ID: 679311 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route,	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 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>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	670311					
	0/9311						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment	A dama an of Toot Conditions	II:-h				
	Metric 16:	Adequacy of Test Conditions	High	and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions described in Table 2.			
	Metric 17:	Outcome Assessment Methodology	Medium	Survivor count determined after the 10 day exposure but not reported as percent mortal- ity.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted at conclusion of 10 day exposure.			
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	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	vsis					
	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	Survival data reported in Table 4 and LC50 values shown in Figure 1 and Table 6.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability not reported but results suggest no excessive variability within replicates.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

HERO ID: 679311 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media, Path:	Call, D. J., C Parkerton, T exposures. E Overall Dura Aquatic (fre	Cox, D. A., Geiger, D. L., Genisot, K. I., M 2. F., Reiley, M. C., Ankley, G. T., Mount, Environmental Toxicology and Chemistry 2 ation: 4 - 10 days; Exposure Duration: 4 - shwater); Sediment; Not determined by stu	arkee, T. P., B D. R. (2001). 20(8):1805-18 10 days 10y authors (i	rooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment 815. e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate: Developmen Dibutyl phth 679311	Arthropods; <i>Hyalella azteca</i> ; Not Applica t/Growth alate (DBP)	able (e.g., fun	gi or algae studies) or Not Reported
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified as a "single isomer" and the identify, including CASRN was referenced in an outside paper, Call et al 2001.
	Metric 2:	Test Substance Source	High	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 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	Biological response of control groups was appropriate as shown in Table 4.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	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 Administration	High	Exposure consistency was reported and consistent among different DBP treatments and controls.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in methods and the cited refer- ence (Call et al 2001).
	Metric 10:	Exposure Duration and Frequency	High	The 10-day exposure duration was appropriate for the experimental design.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were acceptable and spanned five concentrations per test species in addition to the control. Nominal doses were unclear, however measured doses were reported in sediment and pore water.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via sediment.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	Source of test organisms was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to the exposure was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized three replicates of five different concentrations with 10 organ- isms per beaker, three sediment control replicates with 10 test organisms per beaker, and three silica sand control replicates with 10 test organisms per beaker.

Domain 5: Outcome Assessment

Environmental Hazard Evaluation

HERO ID: 679311 Table: 2 of 2

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route	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 Aquatic (freshwater): Sediment: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media, Path:	riquite (ire.						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
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, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions were described in Table 2.			
	Metric 17:	Outcome Assessment Methodology	High	Sediment was sieved, survivors were collected, dried, and weighed.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was conducted at 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 the study groups in environmental conditions or other factors that could influence the 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	vsis					
	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 4. Results were represented as aver- age dry weight per individual.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported, but results suggest no excessive variability within repli- cates.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	High				

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Call, D. J., I F., Reiley, M Environmen Overall Dura Aquatic (fre Invertebrate: Mortality Dibutyl phth 679312	Markee, T. P., Geiger, D. L., Brooke, L. <i>A. C.</i> , Ankley, G. T., Mount, D. R. (2001) tal Toxicology and Chemistry 20(8):1798- ation: 4 - 10 days; Exposure Duration: 4 - shwater); Water; Not determined by study ; Arthropods; <i>Hyalella azteca</i> ; Juvenile nalate (DBP)	 Γ., Vandevent An assessn 1804. 10 days authors (i.e., 	er, F. A., Cox, D. A., Genisot, K. I., Robillard, K. A., Gorsuch, J. W., Parkerton, T. ent 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)
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, but no structure or CASRN were given.
	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				
	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	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 Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
	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	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	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	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 Assessment

Environmental Hazard Evaluation

HERO ID: 679312 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Call, D. J., J F., Reiley, M Environmen	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.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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:	Invertebrate; Arthropods; Hyalella azteca; Juvenile						
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	679312						
Domain		Metric	Rating	Comments			
	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					
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 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	Mean 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 LC50 fo	or Hyalella was reported for DBP as 0.63 m	ıg/L.				
Overall Quali	ty Detern	nination	High				

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (free	or Research Institute, (1997). Sediment tox tion: 4 - 10 days; Exposure Duration: 4 - shwater); Sediment; Not determined by stu	kicity testing 10 days dy authors (i	program for phthalate esters. e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Hyalella azteca; Not Applica	ble (e.g., fun	gi or algae studies) or Not Reported
Health Outcome:	Mortality			
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	7325945			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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				
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	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	High	The experimental system and methods for preparation of test media were described in
	Weate 7.	Preparation	mgn	adequate detail.
	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 using appropriate analytical technologies and
	M 10	Concentration		methods.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.
	Metric 11:	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	High	Exposure concentrations were below the water solubility limit.
Domain 4. Test Ore	~			
Domain 4: Test Organisi	III 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
	Weater 11.	Conditions	mgn	An preneument conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and	Medium	There were 10 organisms per replicate with two replicates 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 maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
		Cont	inued on nex	t page

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Dibutyl Phthalate

HERO ID: 7325945 Table: 1 of 7

		contin	ued from p	previous page			
Study Citation:	Lake Superi	or Research Institute, (1997). Sediment toxi	icity testing	program for phthalate esters.			
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	0 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:							
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	7325945						
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	U				
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.			
Domain /: Data Present	ation and Anal	lysis	TT' 1				
	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	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	High				

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HERO ID: 7325945 Table: 2 of 7

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (free	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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:	Species, Age: Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	7325945							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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								
	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 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 methods for preparation of test media were described in				
	Wette 7.	Preparation	mgn	adequate detail.				
	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 Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.				
	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 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							
	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	There were 10 organisms per replicate with two replicates used.				
		Replicates per Group						
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	0	,, <u>,</u>				

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 2 of 7

		contin	nued from p	revious page			
Study Citation:	Lake Superio	or Research Institute, (1997). Sediment tox	icity testing	program for phthalate esters.			
Duration:	Overall Dura	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:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Hyalella azteca; Not Applica	ble (e.g., fun	gi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
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					
Domain 7. Dua Present	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, but results were described in the tables.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Quali	ty Detern	nination	High				

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HERO ID: 7325945 Table: 3 of 7

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (free	or Research Institute, (1997). Sediment to ation: 4 - 10 days; Exposure Duration: 4 - shwater); Sediment; Not determined by stu	xicity testing 10 days udy authors (i	program for phthalate esters. .e., chemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age:	Faxa, Species, Age: Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mortality				
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	7325945				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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					
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	Medium	The study reported that organisms were randomly allocated into 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 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 appropriate analytical technologies and methods.	
	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	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 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	There were 10 organisms per replicate with two replicates used.	
		Replicates per Group			
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	6		

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 3 of 7

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Lake Superio Overall Dura Aquatic (frea Invertebrate)	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)				
Health Outcome:	Mortality		, (e.g., 141	gi of algue studies) of root reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	7325945					
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	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	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	None					
Overall Quality Determination		High				

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HERO ID: 7325945 Table: 4 of 7

Exposure Route,	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Development Dibutyl phtha	Arthropods; <i>Hyalella azteca</i> ; Not Applica t/Growth alate (DBP)	able (e.g., fun	gi or algae studies) or Not Reported			
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance							
]	Metric 1:	Test Substance Identity	High	Chemical was identified by name, lot # 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 >99%			
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 reported and reasonable			
]	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
Domain 3: Exposure Char	acterization						
Domain 5. Exposure Char	Metric 7	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in			
	Metric 7.	Preparation	Ingn	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	High	Exposure concentrations were measured using appropriate analytical technologies and methods.			
]	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 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 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	all pretreatment conditions were the same for control and exposed organisms			
]	Metric 15:	Conditions Number of Organisms and	Medium	10 per replicate with 2 replicates used			
		Replicates per Group					
Domain 5: Outcome Asses	ssment						
]	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					

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 4 of 7

		contin	ued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Lake Superio Overall Dura Aquatic (frea Invertebrate;	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route) Invertebrate: Arthropods: <i>Hyalella azteca</i> : Not Applicable (e.g., function algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	7325945					
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	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	unexpected outcomes were satisfactorily explained.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	High			

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HERO ID: 7325945 Table: 5 of 7

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superio Overall Dura Aquatic (fre	uperior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. Duration: 4 - 10 days; Exposure Duration: 4 - 10 days c (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; Hyalella azteca; Not Applica	able (e.g., fun	gi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name, lot # 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 >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			
	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	High	The experimental system and methods for preparation of test media were described in			
		Preparation	0	adequate detail			
	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 using appropriate analytical technologies and methods.			
	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 suitable for a dose			
		Spacing of Exposure Levels	0	response			
	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 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 (17	Conditions	м г				
	Metric 15:	Number of Organisms and	Medium	10 per replicate with 2 replicates used			
		Replicates per Group					
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	High	outcomes were assessed consistently across study groups			
		Assessment	0				

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 5 of 7

continued from previous page							
Study Citation: Duration: Exposure Route, Media, Path: Taxa Species Age:	Lake Superi Overall Dur Aquatic (fre	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)					
Health Outcome:	Developmen	t/Growth	, ie (e.g., iui				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	7325945						
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 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 shown for each treatment and control group			
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	High				

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HERO ID: 7325945 Table: 6 of 7

Study Citation: Duration: Exposure Route,	dy Citation:Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.ration:Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 daysposure Route,Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)dia, Path:Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Media, Path:						
Taxa, Species, Age:						
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	ialate (DBP)				
	1323943					
Domain Domain 1: Tost Substan	22	Metric	Rating	Comments		
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	The chamical was identified by name lot # and CAS#		
	Metric 2:	Test Substance Source	Low	The electrical was identify was NOT analytically verified by the performing laboratory		
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as $>99\%$		
	Wette 5.	Test Substance I unity	Ingn	chemical purity was reported as >>> %.		
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 reported and reasonable.		
	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	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 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 suitable for a dose		
		Spacing of Exposure Levels	C	response.		
	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. 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	8	t and the second s		
	Metric 15:	Number of Organisms and	Medium	There were 10 organisms per replicate with two replicates used.		
		Replicates per Group				
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 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	0			

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 6 of 7

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Lake Superio Overall Dura Aquatic (frea Invertebrate)	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)				
Health Outcome:	Mortality		, (e.g., 141	gi of algue studies) of root reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	7325945					
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	Hıgh	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 shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	None					
Overall Quality Determination		High				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 7325945 Table: 7 of 7

Exposure Route,	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route)						
Taxa, Species, Age: Health Outcome: Chemical:	Invertebrate; Development Dibutyl phtha	Arthropods; <i>Hyalella azteca</i> ; Not Applica t/Growth alate (DBP)	able (e.g., fun	gi or algae studies) or Not Reported			
HERO ID:	7325945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance							
]	Metric 1:	Test Substance Identity	High	Chemical was identified by name, lot # 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 >99%			
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 reported and reasonable			
]	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
Domain 3: Exposure Char	acterization						
Domain 5. Exposure Char	Metric 7	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in			
	Metric 7.	Preparation	Ingn	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	High	Exposure concentrations were measured using appropriate analytical technologies and methods.			
]	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 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 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	all pretreatment conditions were the same for control and exposed organisms			
]	Metric 15:	Conditions Number of Organisms and	Medium	10 per replicate with 2 replicates used			
		Replicates per Group					
Domain 5: Outcome Asses	ssment						
]	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					

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 7 of 7

		contin	ued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Lake Superio Overall Dura Aquatic (frea Invertebrate;	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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 route) Invertebrate: Arthropods: <i>Hyalella azteca</i> : Not Applicable (e.g., function algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	7325945					
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	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	unexpected outcomes were satisfactorily explained.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	High			

Study Citation:	Jr, May	yer, F., Sanders, H. O., Walsh, D. F. (1973	3). Toxicity, residue dyn	namics, and reproductive effects of phthalate esters in aquatic invertebrates.				
Dunation	Enviror	Environmental Research 6(1):84-90. 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 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:	Invertel	brate; Arthropods; <i>Ischnura verticalis</i> ; Juven	ile					
Health Outcome:	ADME	(biotransformation)						
Chemical:	Dibutyl	l phthalate (DBP)						
HERO ID:	133464	16						
Domain		Metric	Rating	Comments				
Oomain 1: Test Substance								
Ν	fetric 1:	Test Substance Identity	High	The chemical was identified by name.				
Ν	fetric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laborator				
N	Ietric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.				
Domain 2: Test Design								
N	Ietric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.				
Ν	Ietric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.				
Ν	Ietric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Demein 2. Ennerne Cham								
Joinani 5: Exposure Charae	Actuic 7.	Experimental System/Test Media	Low					
14	icult 7.	Preparation	LOW	test concentrations				
Ν	fetric 8:	Consistency of Exposure	Low	Few details of exposure administration were reported and exposures were administer				
1.		Administration	2011	consistently across study groups.				
Ν	Ietric 9:	Measurement of Test Substance	High	Exposure concentrations were measured.				
		Concentration						
Ν	letric 10:	Exposure Duration and Frequency	Hıgh	The duration of exposure and/or exposure frequency were reported and appropriate in the study true.				
N	Antria 11.	Number of Exposure Groups/	Low	Only one treatment was reported				
IV	leure II.	Specing of Exposure Levels	LOW	Only one treatment was reported.				
Ν	fetric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.				
Domain 4: Test Organism	C . 1 10		Ŧ					
N	1etric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.				
Ν	letric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.				
Ν	fetric 15.	Conditions Number of Organisms and	Low	The number of test replicates was not reported				
14	icule 15.	Replicates per Group	Low	The humber of test replicates was not reported.				
Oomain 5: Outcome Assess	sment							
Ν	letric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.				
Ν	letric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.				
		C	ontinued on next page .					

Environmental Hazard Evaluation

HERO ID: 1334646 Table: 1 of 1

		сог	ntinued from previous p	bage				
Study Citation:	Jr, Ma	yer, F., Sanders, H. O., Walsh, D. F. (1973)	. Toxicity, residue dyna	amics, and reproductive effects of phthalate esters in aquatic invertebrates.				
	Enviro	nmental Research 6(1):84-90.						
Duration:	Overall	l Duration: 4 - 10 days; Exposure Duration: 4	- 10 days					
Exposure Route, Media, Path:	Aquatio	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	e: Inverte	Invertebrate; Arthropods; Ischnura verticalis; Juvenile						
Health Outcome:	ADME	ADME (biotransformation)						
Chemical:	Dibuty	Dibutyl phthalate (DBP)						
HERO ID:	133464	16						
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment wer provided.				
main 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 environmenta conditions.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.				
main 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.				
ditional Commonta	None							

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Call, D. J., I F., Reiley, M Environmen Overall Dura Aquatic (free Invertebrate; Mortality Dibutyl phth 679312	Markee, T. P., Geiger, D. L., Brooke, L. T A. C., Ankley, G. T., Mount, D. R. (2001) tal Toxicology and Chemistry 20(8):1798- ation: 4 - 10 days; Exposure Duration: 4 - shwater); Water; Not determined by study Worms (e.g., Annelids, Nematodes); <i>Lum</i> alate (DBP)	F., Vandevent An assessn 1804. 10 days authors (i.e., briculus varia	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. chemical of interest in exposure water, but unable to determine exact uptake route) <i>egatus</i> ; Adult
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce		2	
	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				
	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 Cha	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	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
	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/	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	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism	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:	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

Environmental Hazard Evaluation

HERO ID: 679312 Table: 1 of 1

continued from previous page							
Study Citation:	Call, D. J., J F., Reiley, M Environmen	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.					
Duration:	Overall Dura	ation: 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;	Worms (e.g., Annelids, Nematodes); Lumi	briculus vari	egatus; Adult			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	679312						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.			
	Metric 17:Outcome Assessment MethodologyMetric 18:Consistency of Outcome		High	The outcome assessment methodology reported the intended outcome of interest.			
			High	Outcomes were assessed consistently across study groups.			
		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 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	Mean data for exposure-related findings were shown for each treatment and control			
	Metric 23:	Explanation of Unexpected Outcomes	High	group. There were no unexpected outcomes.			
Additional Comments:	The LC50 fo	or DBP to Lumbriculus was reported as 2.4	8 mg/L.				
Overall Quality Determination High							

Study Citation: Duration: Exposure Route, Media, Path:	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters. 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;	Worms (e.g., Annelids, Nematodes); Lum	briculus vari	egatus; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323943					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, lot # 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						
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 reported and reasonable.		
	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	High	The experimental system and methods for preparation of test media were described in		
	Methe 7.	Preparation	Ingn	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 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 suitable for a dose		
		Spacing of Exposure Levels	C	response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were 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	Medium	There were 10 organisms per replicate with two replicates used.		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
20man 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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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 7325945 Table: 1 of 1

continued from previous page								
Study Citation:	Lake Superio	Lake Superior Research Institute, (1997). Sediment toxicity testing program for phthalate esters.						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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;	Worms (e.g., Annelids, Nematodes); Lumb	oriculus vari	egatus; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	7325945							
Domain		Metric	Rating	Comments				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
Assessment								
Domain 6: Confounding	y / Variable Co	atrol						
Domain 0. Comounding	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	ysis	TT' 1					
	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, but results were described in the tables.							
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.				
Additional Comments:	None							
Overall Quality Determination			High					

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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. 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) Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Cell signaling/function Dibutyl phthalate (DBP) 789598					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	Metric 1: Metric 2: Matria 3:	Test Substance Identity Test Substance Source	Medium High	Chemical was identified by correct nomenclature and chemical structure. CASRN was not reported. Source of DBP was Riedelde Haen Co.		
	Wietric 5.	Test Substance Fullty	Low	Purity was not reported.		
Domain 2: Test Design	Metric 4: Metric 5: Metric 6:	Negative Controls Negative Control Response Randomized Allocation	High High Low	Negative controls were used. Biological responses of controls were appropriate. 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 specifi- cally 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 0.	Administration Massurement of Test Substance	Low	Exposure consistent across groups.		
	Metric 9: Metric 10: Metric 11:	Concentration Exposure Duration and Frequency Number of Exposure Groups/ Spacing of Exposure Levels	Low High Medium	An appropriate exposure time was used. 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	Testing was below the solubility limit as reported. But the authors did not report the concentration of acetone used to dissolve the phthalate and did not measure the phthalate concentration.		

Domain 4: Test Organism

Dibutyl Phthalate

Environmental Hazard Evaluation

continued from previous page					
Study Citation:	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.				
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:	Dibutyl phthalate (DBP)				
HERO ID:	789598				

Domain		Metric	Rating	Comments	
	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.	
	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 test organism 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	sessment				
	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 Assessment	High	Details of the immune function in vitro assays were reported and assessed consistently across groups.	
Domain 6: Confounding	a / Variable Co	atrol			
Domain 0. Comounding	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 across treatment groups.	
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.	
Domain 7: Data Present	Demain 7. Data Procentation and Analysis				
Domain 7. Data Present	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.	
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Dibutyl Phthalate

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Study Citation:	Sung, H. H., Aquatic Tox	Kao, W. Y., Su, Y. J. (2003). Effects and to cology 64(1):25-37.	oxicity of phtha	late esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii.		
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-9	5h)		
Exposure Route,	Aquatic (fre	shwater); Cell Culture Media; Not determine	ed by study aut	hors (i.e., chemical of interest in exposure water, but unable to determine exact		
Media, Path:	uptake route)		······································		
Taxa, Species, Age:	Invertebrate;	Arthropods; Macrobrachium rosenbergii; No	ot Applicable (e	.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-Cell signaling/function					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	789598					
Domain		Metric	Rating	Comments		
	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).		
Additional Comments:	oxide production (Figures 2 and 3). 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) Hemocyte toxicity/viability assays included detection of cell death via necrosis, detection of cell death via apoptosis, and impacts on cellular morphology assessed by microscopy. This form was used to evaluate impacts to the nonspecific cell-mediated immune defense responses due to DBP.					

Overall Quality Determination

Medium

cally mention random allocation of the hemocytes from different prawns for the various

Study Citation:	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.					
Duration:	Overall Dura	ation: 0 - 4 days (0-901); Exposure	Duration: 0 - 4 days (0-90			
Exposure Route,	Aquatic (fre	shwater); Cell Culture Media; Not	determined by study auth	hors (i.e., chemical of interest in exposure water, but unable to determine exact		
Media, Path:	uptake route					
Taxa, Species, Age:	Invertebrate	; Arthropods; Macrobrachium roser	ubergii; Not Applicable (e.	.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic	-Cell signaling/function				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789598					
Domain	Metric Rating Comments					
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Medium	Chemical was identified by correct nomenclature and chemical structure. CASRN was not reported.		
	Metric 2:	Test Substance Source	High	Source of BBP was Aldrich Chem. Co.		
	Metric 3:	Test Substance Purity	Low	Purity was not reported.		

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Domain		Test	Deston
Domain	2.	1050	Design

Metric 4:	Negative Controls	High	Negative controls used.
Metric 5:	Negative Control Response	High	Biological responses of 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 specifi-

Domain 3: Exposure Characterization					
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	Exposure administration was consistent across groups.		
Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not reported/measured.		
Metric 10:	Concentration Exposure Duration and Frequency	High	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	Below solubility limit as reported. But the authors did not report the concentration of acetone used to dissolve the phthalate and did not measure the phthalate concentration.		
Domain 4: Test Organism					
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.		
Continued on next page					

assays.

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Dibutyl Phthalate

		continued from previous page				
Study Citation:	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). E Aquatic Toxicology 64(1):25-37.	ffects and toxicity of phthalate esters to l	nemocytes of giant freshwater prawn, Macrobrachium rosenbergii.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposur	re Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Cell Culture Media; N	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 ros	enbergii; Not Applicable (e.g., fungi or al	gae studies) or Not Reported			
Health Outcome:	Mechanistic-Cell signaling/function					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	789598					
Domain	Metric	Rating	Comments			

Domain		Metric	Rating	Comments
Metr	ric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 8C 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 test organism which can affect the immune system. The exposure to ph- thalates was in vitro using hemocytes (immune cells) isolated from prawns.
Metr	ric 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 Assessme	ent			
Metr	ric 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.
Metr	ric 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].
Metr	ric 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: Confounding / Vari	able Cor	ntrol		
Metr	ric 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.
Metr	ric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain /: Data Presentation a	ind Anal	ysis Statistical Matheda	Law	Charles and a second state of the second state
Metr	ric 21:	Statistical Methods Benerting of Data	LOW	Statistical analysis was reported but not explained in detail.
Metr	nc 22:	Keporung 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.
Metr	ric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report SE, SD, CI or such variability details for the cell death (necrosis and apoptosis) and cell morphology data.

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Environmental Hazard Evaluation

HERO ID: 789598 Table: 2 of 2

continued from previous page						
Study Citation:	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.					
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:	Dibutyl phthalate (DBP)					
HERO ID:	789598					
Domain	Metric	Rating	Comments			
Additional Comments:	This study was an in vitro experiment where rosenbergii) were exposed to BBP, DBP, DE viability assays: (1) Nonspecific cell-mediat (a measure of the initial procedures of either functions), and nitroblue tetrazolium solutio toxicity/viability assays included detection of microscopy. This form was used to evaluate	e hemocytes (immune cells) isolated from t EHP, or DCHP. Endpoints encompassed non ed immune defense response assays include r phagocytosis or encapsulation), phenoloxi on (NBT) assay to determine superoxide pro of cell death via necrosis, detection of cell de hemocyte toxicity/viability due to DBP.	he hemolymph of 5 to 10 giant freshwater prawn (Macrobrachium specific cell-mediated immune function assays as well as hemocyte d determination of hemocytic adhesion and pseudopodia formation dase activity assay (a measure of pathogen recognition and defense eduction (a measure of highly microbicidal activity); (2) Hemocyte eath via apoptosis, and impacts on cellular morphology assessed by			

Overall Quality Determination

Medium

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: Exposure Route, Media, Path:	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; Arthropods; Orconectus nais; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 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	Purity and/or grade of 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 group 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				
	Metric 7:	Experimental System/Test Media Preparation	Low	Concentrations of the test substance were not measured during the study.	
	Metric 8:	Consistency of Exposure Administration	Medium	Some 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 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 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 Organism					
2 ontain 1. rost 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 Replicates per Group	Low	The number of test replicates was not reported.	
		· ····································			

Domain 5: Outcome Assessment

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 1334646 Table: 1 of 1

continued from previous 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 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; Orconectus nais; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mortality	Mortality			
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	1334646				
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 the outcome assessment were not reported	
		Assessment		not reported.	
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	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 Quality Determination

Uninformative

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:	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: Health Outcome:	Invertebrate; Arthropods; <i>Paleomonetes kadiakensis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)			
HERO ID:	1334646	1334646			
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	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design					
6	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.	
	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	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	Domain J. Outcome Assessment Matria 16. Adaptacy of Test Conditions				
	Metric 17:	Autquacy of rest Conditions	Low Medium	The outcome assessment methodology was not clearly reported	
	wienie 17.	Sucome Assessment Methodology	Wiculuiii	The outcome assessment methodology was not clearly reported.	
			Continued on next page		

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			unucu ironi previou	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.							
D	Environmen	Environmental Research 6(1):84-90.						
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 auth	ors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Paleomonetes kadiakensis; Not A	Applicable (e.g., fungi	or algae studies) or Not Reported				
Health Outcome:	ADME (biot	ransformation)						
Chemical:	Dibutyl phth	alate (DBP)						
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 wer				
		Assessment		provided.				
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 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 Commentar	None							

Study Citation: Duration:	Adams, W. J organisms. E Overall Dura	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)						
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)				
Media, Path:								
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	Mortality Dibutul phthelete (DBD)						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	1321996							
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce		-					
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature was reported without CASRN.				
	Metric 2:	Test Substance Source	Low	The source was reported as provided by manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.				
	Metric 3:	Test Substance Purity	High	The chemical was at least 95% pure.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5: Negative Control Response High The control response w		The control response was acceptable.					
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to pre- vent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	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 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 exposure were appropriate for this 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 water solubility.				
Domain 4. Test Organis	~							
Domain 4: Test Organisi	Metric 13.	Test Organism Characteristics	Low	A source was not reported				
	Metric 14	Acclimatization and Pretreatment	High	An appropriate acclimation for the test was reported				
	1,10010 1 4 .	Conditions	mgn	in appropriate accontiation 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 ...

HERO ID: 1321996 Table: 1 of 1

	continued from previous 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:	Invertebrate;	Arthropods; Paratanytarsus parthenogene	<i>etica</i> ; Not Ap	plicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
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 Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.			
		Design and Procedures	-				
	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 Frederik	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.			
	Wieule 25.	Explanation of Onexpected Outcomes	Ingli	to unexpected outcomes were reported.			
Additional Comments:	None						
Overall Quali	Overall Quality Determination		High				

Study Citation: Duration: Exposure Route, Media, Path:	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) 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; Paratanytarsus parthenogeni	ica; Larvae				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
	1310219						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT' 1				
	Metric 1:	Test Substance Identity	High	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	Purity was reported as 100% active ingredient.			
Domain 2. Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	Negative controls were used			
	Metric 5:	Negative Control Response	High	Percent mortality for controls was shown in Table 3			
	Metric 6:	Randomized Allocation	Medium	Organisms were impartially distributed into the test vessels.			
Domain 3: Exposure Ch	aracterization						
-	Metric 7:	Experimental System/Test Media Preparation	High	Static test conditions were described in detail.			
	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	Test concentrations were verified analytically as shown in Table 1 and described in Appendix I.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported and adequate (mortality reported at 48hr and 24hr).			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five concentrations of DBP were tested spanning approximately one order of magnitude between the highest and lowest concentration.			
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were approximately at or below the solubility limit.			
		· · · · ·		· · · · · · · · · · · · · · · · · · ·			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Paratanytarsus parthenogenica were 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 organisms were acclimatized.			
	Metric 15.	Conditions Number of Organisms and	Low	Three replicate beakers per concentration with five midge larvae per beaker			
	Methe 15.	Replicates per Group	Low	The replicate beakers per concentration with five intege fail var per beaker.			
		at and the search					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described, reported and 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 nex	at page			

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		contin	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Bionomics,, Overall Dura Aquatic (fres	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) 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	Mortality	Annopous, 1 araianyiarsus parmenogena	<i>u</i> , Lai vac			
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1316219					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Mortality assessment was conducted at 24 and 48hr and appeared to be consistent		
	Assessment among study groups.					
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test	High	No differences were reported.		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure (e.g., in- fection) were reported for each study group and 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	The LC50 at 48 hr exposure was determined via moving angle analysis as described in the footnote of Table 4.		
	Metric 22:	Reporting of Data	High	Outcomes were described in Table 3 and LC50 values were shown in Table 4.		
	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 also reported but unclear how authors obtained (or estimated) LC50 values based on a limit test					
Overall Quali	ty Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kuang, Q. J.	Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-					
Duration: Exposure Route, Media, Path:	608. Overall Dura Aquatic (fre	608. 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:	Vegetation; Non-vascular Plants; <i>Algae</i> ; natural algae; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP)						
Domain	1332820	Metric	Rating	Comments			
Domain 1: Test Substand	ce		0				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	It was reported the DBP was manufactured in Shanghai, China but this is not enough information on the source. It was not reported if the DBP was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.			
Domain 2: Test Design	Matria A.	Negative Controls	I				
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control. It was reported that a vehicle solvent was used, but it was unclear if the control was a solvent control.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and reported in Table 2.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.			
Domain 3. Exposure Ch	aracterization						
2 oniun of 2npoone on	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were reported on the preparation of the test media and concentrations of test substance were not measured during the study. The solvent concentration was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported. The test chambers were 50L buck- ets open to conditions. The temperatures were reported to be between 16-24C, which could lead to variation.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be seven days, which is longer than is typical for acute toxicity studies with algae.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four exposure groups plus a control, and the spacing of the exposure levels was adequate for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	Low	It was reported that a vehicle solvent was used, but it was unclear if the control was a solvent control. The solvent concentration was not reported.			
Domain 4: Test Organisi	n						
	Metric 13:	Test Organism Characteristics	Low	It was reported that the algae was obtained from East Lake in Wuhan, China. This leaves doubt about the health of the organisms.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the algae was acclimated to the test conditions prior to the start of the test.			
		Cont	tinued on nex	t page			

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Environmental Hazard Evaluation

HERO ID: 1332820 Table: 1 of 1

		conti	nued from p	revious page		
Study Citation:	Kuang, Q. J.	, Zhao, W. Y., Cheng, S. P. (2003). Toxicity	of dibutyl p	hthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-		
Duration: Exposure Route, Media, Path:	608. 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:	Vegetation; 1 Developmen Dibutyl phth	Non-vascular Plants; <i>Algae</i> ; natural algae; l t/Growth alate (DBP)	Not Applicat	ble (e.g., fungi or algae studies) or Not Reported		
Domain	1332820	Metric	Rating	Comments		
Domain	Metric 15:	Number of Organisms and Replicates per Group	Low	Initial cell counts for each exposure and control were reported in Table 2. Study authors did not report the number of replicates.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 16-24C in 50L buckets with a natural photoperiod length that was not elaborated on. It is unclear if any sort of algal media was used. It was reported that lake water was used in the study.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal density-development/growth.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were not clearly reported but outcomes were assessed consistently across study groups. Cell counts were performed with a 0.1mm^3 plankton counting chamber under a microscope every two days.		
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. It was not reported if the algae was 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 Present	ation and Anal	vsis				
Domain / Data Present	Metric 21:	Statistical Methods	Low	Study authors did not report conducting statistical analysis, but an independent statistical analysis could be conducted from the data provided in Table 2.		
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response is reported in Table 2. Exposure related findings are also presented in Figure 3.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This portion concentratio found in the	of the evaluation was on the acute toxicinns, so development and growth was selected lake. The paper listed several genera that v	ty of DBP of d as the outco vere found, b	n natural algae obtained from East Lake in Wuhan, China. The study measured cell ome of interest. Algae was selected as the taxa, as the study was on mixed algae species out the results did not elaborate on any genera.		
Overall Quali	ty Detern	nination	Low			

Study Citation:	Melin, C., I	Egneus, H. (1983). Effects of di-n-butyl p	hthalate on growth and p	photosynthesis in algae and on isolated organelles from higher plants.			
·	Physiologia	Plantarum 59(3):461-466.	C 1				
Duration:	Overall Dura	ation: 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., chemical of in	terest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Chlorella emersonii; CCAP strain 211/8h; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mechanistic-Photosynthesis						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1333016						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
		ý					
Domain 2: Test Design							
_	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control, which was a solvent control con-			
	Matria 5.	Nagativa Control Bagnanga	High	tanning ethanol.			
	Matria 6	Dendemized Allocation	Law	It menerates the stand beaution and a stand stand stand stands and a second			
	Metric 0:	Randomized Anocation	LOW	It was not reported now the argae was anocated into study groups.			
Domain 2: Exposure Ch	araatarization						
Domain 5. Exposure Ch	Matria 7:	Experimental System/Test Media	Low	The systems provided limited details on the properties of the DDD test solution			
	Methe 7.	Propagation	LOW	The autions provided infined details on the preparation of the DBF test solution.			
	Matric 8.	Consistency of Exposure	Low	Details regarding the exposure administration ware limited. Study outhers reported the			
	Methe 8.	A dministration	LOW	temperature and test volumes though			
	Metric 9.	Measurement of Test Substance	Low	Study authors did not report if the test concentrations were measured at any point in the			
	Metric 9.	Concentration	Low	study autors did not report if the lest concentrations were measured at any point in the			
	Metric 10 [.]	Exposure Duration and Frequency	Low	It was reported that the algae were exposed for 40 minutes			
	Metric 11:	Number of Exposure Groups/	Uninformative	The exposure levels for the test on C emersonic were not reported			
	metric 11.	Spacing of Exposure Levels	emmonnative	The exposure levels for the lest on C. entersonn were not reported.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the algae was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.			
	Metric 15.	Conditions Number of Organisms and	Low	The algae concentration and the number of replicates was not reported for this part of			
	Methe 15.	Replicates per Group	LOW	the study			
		Replicates per Group		· · · · · · · · · · · · · · · · · · ·			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Egneus and Blanck 1977 were cited for the culturing techniques, but details were limited			
		·1·····		in the paper.			
		<u> </u>	ontinued on next nego				
		t	ontinueu on next page.	••			

PUBLIC RELEASE DRAFT May 2025

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333016 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation:	Melin, C., E Physiologia	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466					
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 auth	ors (i.e., chemical of	interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; 1	Non-vascular Plants; Chlorella emersonii; CCA	AP strain 211/8h; Not	Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	-Photosynthesis					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333016						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–CO2 -dependent oxygen evolution as a measurement of photosynthesis.			
	Metric 18:	Consistency of Outcome Assessment	Low	Limited details were provided on the protocol for outcome assessment.			
Domain 6: Confoundin	g / Variable Coi	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmenta conditions or other non-treatment-related factors across study groups. It was not re- ported if the algae was 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 the exposure.			
Domain 7: Data Presen	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Growth curve determination and IC50 value determination were described in the text.			
	Metric 22:	Reporting of Data	High	Exposure data and control response were reported in Figure 2.			
	Matria 22.	Explanation of Unexpected Outcomes	High	Variability was reported in Figure 2.			

Overall Quality Determination

Uninformative

Study Citation:	Melin, C., I	Egneus, H. (1983). Effects of di-n-butyl pl	nthalate on growth and p	photosynthesis in algae and on isolated organelles from higher plants.				
	Physiologia	Plantarum 59(3):461-466.						
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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:	Vegetation;	Vegetation: Non-vascular Plants: Chlorella emersonii: CCAP strain 211/8h; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	1333016							
	1000010							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice		_					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control, which was a solvent control con- taining ethanol.				
	Metric 5:	Negative Control Response	Low	The negative control response was not reported for C. emersonii.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.				
Domain 3: Exposure Ch	naracterization							
Domain 5. Exposure of	Metric 7.	Experimental System/Test Media	Low	The authors provided limited details on the preparation of the DBP test solution				
	incure /.	Preparation	E0.	The address provided minied details on the propulation of the DDT test solution.				
	Metric 8:	Consistency of Exposure	Low	Details regarding the exposure administration were limited. Study authors reported the				
	intenne or	Administration	2011	temperature and test volumes though.				
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test concentrations were measured at any point in the				
		Concentration		study.				
	Metric 10:	Exposure Duration and Frequency	Low	Study authors did not explicitly state the duration of the study, but Figure 1 indicates it was seven days.				
	Metric 11:	Number of Exposure Groups/	Uninformative	The exposure levels for the growth test on C. emersonii were not reported. It was re-				
		Spacing of Exposure Levels		ported that the results were similar to the S. capricornutum, but it was unclear if the				
				same exposure levels were used.				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Low	The source of the algae was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.				
	M + 1 17	Conditions						
	Metric 15:	Number of Organisms and	Medium	It was reported that the initial algal density was 0.1ug chlorophyll/mL. There were three				
		Replicates per Group		culture hasks per concentration.				

Domain 5: Outcome Assessment

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PUBLIC RELEASE DRAFT May 2025

Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333016 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation:	Melin, C., H Physiologia	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.					
Duration:	Overall Dura	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:	Vegetation; 1	Non-vascular Plants; Chlorella emersonii; CCA	AP strain 211/8h; Not	Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333016						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Egneus and Blanck 1977 were cited for the culturing techniques, but details were limited in the paper.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal growth.			
	Metric 18:	Consistency of Outcome Assessment	Low	It was reported that algae growth was determined by measuring changes in transmittance at 729nm, but methods and instruments used were not reported.			
Domain 6: Confounding	/ Variable Co	ntrol					
Domain 0. Comountaing	Metric 10:	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. It was not re- ported if the algae was 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 the exposure.			
Domain 7: Data Present	ation and Anal	veie					
Domain 7. Data Present	Metric 21.	Statistical Methods	High	Growth curve determination and IC50 value determination were described in the text			
	Metric 22.	Reporting of Data	Low	Data was reported in the text, but continuous data was not presented			
	Metric 22.	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability			
	metric 23.	Explanation of Onexpected Outcomes	LOW	The study and not report any incasures of variability.			
Additional Comments:	This portion This portion reported.	This portion of the evaluation is on the effect of DBP on the growth of algae C. emersonii. Development/growth was selected as the outcome of interest. This portion of the study was rated unacceptable due to the lack of information regarding exposure groups and spacing; test concentrations were not					

Overall Quality Determination

Uninformative

Study Citation:	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	re Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Cell Culture Media; N	ot 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 pyrenoidosa; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5433509					
Domain	Metric	Rating	Comments			

Domain		Metric	Rating	Comments		
Domain 1: Test Substand	се					
	Metric 1:	Test Substance Identity	Medium	The test substance was identified by name in the paper. The supplementary material had information regarding the structure and other physical characteristics.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Sino-pharm Chemical Reagent Co. Ltd. in Nanjing, China, but it was not reported if it was analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99% pure.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 5 and 6 as well as in the supple- mental material.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The study occurred in flasks. The test solution was prepared by creating a stock solution in which DBP was dissolved into acetone for a concentration of 100g/L. This was then diluted to the proper test concentrations using BG-11 algae growth medium.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures occurred in 500mL flasks for 96h using BG-11 growth media. All tests were started with the same initial algal cell count.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is typical of an algae growth study.		
	Metric 11:	Number of Exposure Groups/	High	There were 5 exposure levels, and the spacing was adequate to observe a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	Acetone was reported to be the vehicle solvent, but it was unclear if the negative control was a solvent control.		
Domain 4: Test Organis	m					
<u>c</u> at a	Metric 13:	Test Organism Characteristics	High	Both algal species were from the Freshwater Algae Culture Collection at the Chinese Academy of Sciences.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated at any point.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	S. obliquus initial concentrations were reported to be 2.02 x 10^6 cells/mL, and each concentration was performed in triplicate.		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 5433509 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation:	Gu, S., Zhei Aquatic Tox	ng, H., Xu, Q., Sun, C., Shi, M., Wang, Z icology 191:122-130.	., Li, F. (201	7). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
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)					
Taxa, Species, Age:	Vegetation; 1	Non-vascular Plants; Chlorella pyrenoidosa	a; Not Applic	cable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	Oxidative stress (including redox biology)	-Photosynthe	esis			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5433509						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The algae was cultured in BG-11 algal medium with a 12L:12D photoperiod at 24C and 8000lux.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-photosynthesis and oxidative stress.			
	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	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	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "statistical analysis" section of the paper.			
	Metric 22:	Reporting of Data	High	Control and exposure responses were reported in Figures 5 and 6 as well as in the sup- plemental material. Results were adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures.			
Additional Comments:	This portion of the evaluation was on the effect of DBP on photosynthetic pigments and oxidative stress. Mechanistic outcomes were selected. Data for the photosynthesis outcomes in the paper were not provided because the Figure 4 in the paper is the incorrect figure. There are some data in the supplemental material regarding the photosynthesis outcomes. Supplemental material was not included in the Distiller download, but was found via the link in the article						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae.
	Aquatic Toxicology 191:122-130.
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:	Vegetation; Non-vascular Plants; Chlorella pyrenoidosa; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	5433509

Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Medium	The test substance was identified by name in the paper. The supplementary material had information regarding the structure and other physical characteristics.
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Sino-pharm Chemical Reagent Co. Ltd. in Nanjing, China, but it was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 1 and 3 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.
Domain 3: Exposure Ch	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The study occurred in flasks. The test solution was prepared by creating a stock solution in which DBP was dissolved into acetone for a concentration of 100g/L. This was then diluted to the proper test concentrations using BG-11 algae growth medium.
	Metric 8:	Consistency of Exposure Administration	High	All exposures occurred in 500mL flasks for 96h using BG-11 growth media. All tests were started with the same initial algal cell count.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is typical of an algae growth study.
	Metric 11:	Number of Exposure Groups/	High	There were five exposure levels, and the spacing was adequate to observe a dose re-
	Matria 12.	Spacing of Exposure Levels	Madium	sponse.
	Meuric 12.	Testing at of Below Solubility Limit	Meuluiii	was a solvent control.
Domain 4: Test Organisi	n			
	Metric 13:	Test Organism Characteristics	High	Both algal species were from the Freshwater Algae Culture Collection at the Chinese Academy of Sciences.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated at any point.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	S. obliquus initial concentrations were reported to be 2.02 x 10 ⁶ cells/mL, and each concentration was performed in triplicate.

Domain 5: Outcome Assessment

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route,	Gu, S., Zher Aquatic Toxi Overall Dura Aquatic (free	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130. 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				
Media, Path:	uptake route)				
Taxa, Species, Age: Health Outcome:	Vegetation; I	Non-vascular Plants; <i>Chlorella pyrenoidosa</i> t/Growth	<i>i</i> ; Not Applic	cable (e.g., fungi or algae studies) or Not Reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5433509					
Domain		Metric Rating Comments				
	Metric 16:	Adequacy of Test Conditions	High	The algae was cultured in BG-11 algal medium with a 12L:12D photoperiod at 24C and 8000lux.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-cell density.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Samples were taken at 0, 24, 48, 72, and 96h and counted with a hemocytometer.		
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 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 methods were described in the "statistical analysis" section of the paper.		
	Metric 22:	Reporting of Data	High	Control and exposure responses were reported in Figures 1 and 3 and were adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 1.		
Additional Comments:	This portion selected as the	of the evaluation was on the effect of DB ne outcome of interest.Supplemental mater	P in the cell ial was not ir	density and morphology of the algae C. pyrenoidosa. Development and growth was included in the Distiller download, but was found via the link in the article.		

Overall Quality Determination

High

Study Citation:	Chi, J., Li, B Journal of F	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) days	42(2).179-105.					
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: Health Outcome:	Vegetation;	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Chemical	Dibutyl phth	nalate (DBP)							
HERO ID:	679344								
Domain		Metric	Rating	Comments					
Domain 1: Test Substar	ice								
	Metric 1:	Test Substance Identity	High	The chemical was identified by name as dibutyl phthalate (DBP).					
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma.					
	Metric 3:	Test Substance Purity	High	The test substance purity was reported as 99%.					
Domain 2. Test Design									
2011an 21 1000 2005gu	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. They did not use a negative control without test substance.					
	Metric 5:	Negative Control Response	Medium	Baseline control responses were noted on graphs and in equations.					
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.					
	, . <i>.</i> .								
Domain 3: Exposure Cl	Matric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare					
	Methic 7.	Preparation	LOW	test concentrations					
	Metric 8:	Consistency of Exposure	High	Exposures were consistently administered to test organisms.					
	Metric 9:	Administration Measurement of Test Substance	Medium	Concentrations were measured but only reported as log values on a graph.					
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency differed significantly from typical study designs, and concentrations never reached steady state.					
	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	Metric 12.	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					
	Meule 14.	Conditions	LOW	The study and not report whether test organisms were adeliniatized.					
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.					

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

continued from previous page							
Study Citation:	Chi, J., Li, B	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, Media, Path:	Journal of En Overall Dura Aquatic (free	Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 42(2):179-183. 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:	Vegetation; I	Non-vascular Plants; Chlorella vulgaris; Not	Applicable (e.g., fungi o	or algae studies) or Not Reported			
Health Outcome:	ADME (biot	ransformation)					
Chemical: HERO ID:	Dibutyl phth 679344	alate (DBP)					
Domain		Metric	Rating	Comments			
	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.			
	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 High Details of the outcome assessment protocol were reported, and outcomes were 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 environme or other factors that could influence the outcome assessment.			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 Presentation and Analysis							

	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments:	This was a bi	oconcentration/bioaccumulation study, with va	ariable nitrogen and	phosphorus ratios.

Statistical Methods

Reporting of Data

Overall Quality Determination

Metric 21:

Metric 22:

Uninformative

N/A

High

Statistical analysis was not possible as only one test concentration was used.

Data for exposure-related findings were presented for each treatment over time.

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	and Chemist	try 22(12):3037-3043. ation: 0 - 4 days (0-96h): Exposure Duration	: 0 - 4 days (0-96h)					
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:				······································				
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:	Dibutyl phth	nalate (DBP)						
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, structure were reported.				
	Metric 2:	Test Substance Source	High	DBP 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 >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 control group was reported. The control group had a growth rate 1.6-1.8/ d during the 72 hours 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 Cr	Matric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately proper				
	Metric 7.	Preparation	Low	test concentrations and/or minimize loss of test substance before and during the expo- sure.				
	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 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. An algal growth inhibition test was conducted for 72 hours.				
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the exposure concentrations and the spacing of exposure				
	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 Organis	m							
0	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.				
		C	continued on next page .					

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Dibutyl Phthalate

HERO ID: 789536 Table: 1 of 1

		cont	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	Dverall 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:	Vegetation;	Non-vascular Plants; Pseudokirchneriella subco	<i>apitata</i> ; Not Applicat	le (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	nt/Growth	1 / 11					
Chemical:	Dibutyl phth	nalate (DBP)						
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 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 refrence (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 24hrs to determine growth rates.				
Domain 6: Confounding	g / Variable Co	ntrol Conformating Variables in Test	T					
	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 on outcomes unrelated to exposure.				
Domain 7: Data Procon	tation and Anal	lyreig						
Domain 7. Data Presen	Metric 21.	statistical Methods	High	Nonlinear regression analysis was conducted to estimate EC values and confidence				
	Wietife 21.	Statistical Methods	nigii	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 an of provided for each of the treatment groups and	d control response w l 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: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Gu, S., Zher Aquatic Tox: Overall Dura Aquatic (free uptake route Vegetation; I Mechanistic- Dibutyl phth 5433509	 Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130. 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) Vegetation; Non-vascular Plants; <i>Scenedesmus obliquus</i>; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 5433509 					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Medium	The test substance was identified by name in the paper. The supplementary material had information regarding the structure and other physical characteristics.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Sino-pharm Chemical Reagent Co. Ltd. in Nanjing, China, but it was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99% pure.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 5 and 6 as well as in the supple- mental material.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
2 onium of 2nposine on	Metric 7:	Experimental System/Test Media Preparation	High	The study occurred in flasks. The test solution was prepared by creating a stock solution in which DBP was dissolved into acetone for a concentration of 100g/L. This was then diluted to the proper test concentrations using BG-11 algae growth medium.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures occurred in 500mL flasks for 96h using BG-11 growth media. All tests were started with the same initial algal cell count.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is typical of an algae growth study.			
	Metric 11:	Number of Exposure Groups/	High	There were 5 exposure levels, and the spacing was adequate to observe a dose response.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Acetone was reported to be the vehicle solvent, but it was unclear if the negative control was a solvent control.			
Domain 4: Test Organisi	n						
C	Metric 13:	Test Organism Characteristics	High	Both algal species were from the Freshwater Algae Culture Collection at the Chinese Academy of Sciences.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated at any point.			

Conditions
Number of Organisms and
Replicates per GroupMediumS. obliquus initial concentrations were reported to be 2.02 x 10^6 cells/mL, and each
concentration was performed in triplicate.

Continued on next page ...

Metric 15:

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Environmental Hazard Evaluation

HERO ID: 5433509 Table: 1 of 2

		contin	nued from p	revious page				
Study Citation:	Gu, S., Zher	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae.						
Duration:	Overall Dura	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:	Vegetation; 1	Non-vascular Plants; Scenedesmus obliquus	s; Not Applie	cable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mechanistic	Oxidative stress (including redox biology)	-Photosynthe	esis				
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5433509							
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	The algae was cultured in BG-11 algal medium with a 12L:12D photoperiod at 24C and 8000lux.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-photosynthesis and oxidative stress.				
	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	r / 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				
		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						
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "statistical analysis" section of the paper.				
	Metric 22:	Reporting of Data	High	Control and exposure responses were reported in Figures 5 and 6 as well as in the sup- plemental material. Results were adequate for the outcomes of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures.				
Additional Comments:	Additional Comments: This portion of the evaluation was on the effect of DBP on photosynthetic pigments and oxidative stress. Mechanistic outcomes were selected. Data for the photosynthesis outcomes in the paper were not provided because the Figure 4 in the paper is the incorrect figure. There are some data in the supplemental material regarding the photosynthesis outcomes. Supplemental material was not included in the Distiller download, but was found via the link in the article.							
Overall Qualit	ty Detern	nination	High					

Study Citation:	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae.
	Aquatic Toxicology 191:122-130.
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:	Vegetation; Non-vascular Plants; Scenedesmus obliquus; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	5433509

Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	Medium	The test substance was identified by name in the paper. The supplementary material had information regarding the structure and other physical characteristics.
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Sino-pharm Chemical Reagent Co. Ltd. in Nanjing, China, but it was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 1 and 2 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.
Demein 2. Ernerum Ch				
Domain 5: Exposure Ch	Matria 7.	Experimental System/Test Madia	Illah	The state assumed in the factor. The factor lation many and has mostly a state should be
	Weuric 7:	Preparation	nigii	in which DBP was dissolved into acetone for a concentration of 100g/L. This was then diluted to the proper test concentrations using BG-11 algae growth medium.
	Metric 8:	Consistency of Exposure Administration	High	All exposures occurred in 500mL flasks for 96h using BG-11 growth media. All tests were started with the same initial algal cell count.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is typical of an algae growth study.
	Metric 11:	Number of Exposure Groups/	High	There were five exposure levels, and the spacing was adequate to observe a dose re-
	M + 1 10	Spacing of Exposure Levels		sponse.
	Metric 12:	lesting at or Below Solubility Limit	Medium	Acetone was reported to be the vehicle solvent, but it was unclear if the negative control was a solvent control.
Domain 4: Test Organisi	n			
	Metric 13:	Test Organism Characteristics	High	Both algal species were from the Freshwater Algae Culture Collection at the Chinese Academy of Sciences.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated at any point.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	S. obliquus initial concentrations were reported to be 2.02 x 10 ⁶ cells/mL, and each concentration was performed in triplicate.

Domain 5: Outcome Assessment

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Dibutyl Phthalate

		contir	ued from p	revious page				
Study Citation: Duration: Exposure Route, Media, Path:	Gu, S., Zher Aquatic Toxi Overall Dura Aquatic (fres uptake route)	Gu, S., Zheng, H., Xu, Q., Sun, C., Shi, M., Wang, Z., Li, F. (2017). Comparative toxicity of the plasticizer dibutyl phthalate to two freshwater algae. Aquatic Toxicology 191:122-130. 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						
Taxa, Species, Age:	Vegetation; N	Non-vascular Plants; <i>Scenedesmus obliquus</i>	; Not Applic	cable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5433509							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	High	The algae was cultured in BG-11 algal medium with a 12L:12D photoperiod at 24C and 8000lux.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-cell density.				
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Samples were taken at 0, 24, 48, 72, and 96h and counted with a hemocytometer.				
Domain 6: Confounding	y / 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.				
Domain 7: Data Present	ation and Anal	ysis						
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "statistical analysis" section of the paper.				
	Metric 22:	Reporting of Data	High	Control and exposure responses were reported in Figures 1 and 2 and were adequate for the outcome of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 1.				
Additional Comments:	This portion as the outcor	of the evaluation was on the effect of DBP ne of interest.Supplemental material was no	in the cell de	ensity and morphology of the algae S. obliquus. Development and growth was selected n the Distiller download, but was found via the link in the article.				

Overall Quality Determination

High

Study Citation:	Kuang, Q. J.	Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-						
Duration: Exposure Route, Media. Path:	608. Overall Dura Aquatic (fres	608. 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:	Vegetation; M Developmen Dibutyl phth 1332820	Vegetation; Non-vascular Plants; <i>Scenedesmus obliquus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 1332820						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce	T . A 1	Ŧ					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.				
	Metric 2:	Test Substance Source	Low	It was reported the DBP was manufactured in Shanghai, China but this is not enough information on the source. It was not reported if the DBP was analytically verified.				
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control but it was unclear if the control was a solvent control.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and reported in Table 1.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Domain of Dispositio on	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were reported on the preparation of the test media and concentrations of test substance were not measured during the study. The concentration of the solvent (acetone) was not provided.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. Exposures were conducted in 150mL glass flasks with HB-4 media. Temperatures and photoperiod remained consistent throughout the study, and the same algae concentration was used for all test concentrations.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured 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 test with algae.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups plus a control, and the spacing of the exposure levels was adequate for a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	Low	It was reported that a vehicle solvent was used, but it was unclear if the control was a solvent control. The solvent concentration was not reported.				
Domain 4: Test Organis	m							
C	Metric 13:	Test Organism Characteristics	High	The source of the S. obliquus was reported to be the Department of Pathology, the Insti- tute of Hydrobiology, Academia Sinica China.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the algae was acclimated to the test conditions prior to the start of the test.				
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Environmental Hazard Evaluation

HERO ID: 1332820 Table: 1 of 1

		contin	ued from previo	bus page				
Study Citation:	Kuang, Q. J.	Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-						
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: Chemical: HERO ID:	Vegetation; I Developmen Dibutyl phth 1332820	Vegetation; Non-vascular Plants; <i>Scenedesmus obliquus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 1332820						
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study authors reported that a concentration of 5-7 x 10^5cells/mL of algae was used in each test chamber. The test was conducted in triplicate.				
Domain 5: Outcome Ass	sessment							
Domain 5. Outcome 715.	Metric 16:	Adequacy of Test Conditions	High	The test was conducted at 24C with a 14L:10D photo period. The algae was cultured in 150mL flasks with HB-4 algal media.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal density-development/growth.				
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were not clearly reported but outcomes were assessed consistently across study groups. Chlorophyll a was assessed using spectrophotometry at 650nm, and cell counts were done using a hemocytometer every two days, but methodology details were missing.				
Domain 6: Confounding	/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. It was not reported if the algae was 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 Present	ation and Anal	veie						
Domain 7. Data Present	Metric 21:	Statistical Methods	Low	SGMI 1979 was used to determine EC50 values. However, it did not appear that statis- tics were performed on the other data, but independent statistical analysis may be con- ducted.				
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response is reported in Table 1. Exposure related findings are also presented in Figures 1 and 2.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				
Additional Comments:	tional Comments: This portion of the evaluation was on the acute toxicity of DBP on the single celled algae S. obliquus. Algae concentrations were determined over the 96h test period. Development/growth was selected as the outcome of interest. Study authors also reported a chronic toxicity test was performed, but details regarding the study were not reported nor was any data reported, so an evaluation was not done.							
Overall Qualit	ty Detern	nination	Medium					

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Study Citation:	Kuang, Q. J.,	Luang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-						
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) Vegetation; Non-vascular Plants; <i>Scenedesmus obliquus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 1332820						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; N Development Dibutyl phthe 1332820							
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	It was reported the DBP was manufactured in Shanghai, China but this is not enough information on the source. It was not reported if the DBP was analytically verified.				
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.				
Domain 2: Test Design								
C	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control but it was unclear if the control was a solvent control.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was mentioned in the text in comparison to treatments.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were reported on the preparation of the test media and concentrations of test substance were not measured during the study. The concentration of the solvent (acetone) was not provided.				
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. Exposures were conducted in 150mL glass flasks with HB-4 media. Temperatures and photoperiod remained consistent throughout the study, and the same algae concentration was used for all test concentrations.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in the study.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be four weeks for the chronic study.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups plus a control, and the spacing of the exposure levels was adequate for a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	Low	It was reported that a vehicle solvent was used, but it was unclear if the control was a solvent control. The solvent concentration was not reported.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	The source of the S. obliquus was reported to be the Department of Pathology, the Insti- tute of Hydrobiology, Academia Sinica China.				
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the algae was acclimated to the test conditions prior to the start of the test.				
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Environmental Hazard Evaluation

		contin	ued from previo	ous page				
Study Citation:	Kuang, Q. J. 608.	Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602- 608.						
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route, Madia Path	Aquatic (fres	sinwater); water; Not determined by study au	unors (i.e., chem	ical of interest in exposure water, but unable to determine exact uptake route)				
Taxa Snecies Age	Vegetation: N	Non-vascular Plants: Scenedesmus obliquus:	Not Applicable	(e.g. fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth	riot ripplicable	(e.g., rungi of algae station) of not reported				
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1332820							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study authors reported that a concentration of 5-7 x 10^5cells/mL of algae was used in each test chamber. The test was conducted in triplicate.				
Domain 5: Outcome Ass	sessment							
Domain 5. Outcome 715.	Metric 16:	Adequacy of Test Conditions	High	The test was conducted at 24C with a 14L:10D photo period. The algae was cultured in 150mL flasks with HB-4 algal media.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal density-development/growth.				
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were not clearly reported but outcomes were assessed consistently across study groups. Chlorophyll a was assessed using spectrophotometry at 650nm, and cell counts were done using a hemocytometer every two days, but methodology details were missing.				
Domain 6: Confounding	/ Variable Cor	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		conditions. It was not reported if the algae was 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 Presenta	ation and Anal	vsis						
	Metric 21:	Statistical Methods	Low	SGMI 1979 was used to determine EC50 values. However, it did not appear that statis- tics were performed on the other data, but independent statistical analysis may be con- ducted.				
	Metric 22:	Reporting of Data	Low	Results for the chronic exposure were reported in the text only.				
	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 acute toxicity of DBP on the single celled algae S. obliquus. Algae concentrations were determined over the 96h test period. Development/growth was selected as the outcome of interest. Study authors also reported a chronic toxicity test was performed, but details regarding the study were not reported nor was any data reported, so an evaluation was not done. This evaluation was for the four week chronic exposure.							
Overall Ovel4	w Dotor	nination	Modium					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kuang, Q. J., Zhao, W. Y., Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-
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:	Vegetation; Non-vascular Plants; Scenedesmus obliquus; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Cell signaling/function
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	1332820

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	It was reported the DBP was manufactured in Shanghai, China but this is not enough information on the source. It was not reported if the DBP was analytically verified.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control but it was unclear if the control was a solvent control.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was mentioned in the text in comparison to treatments.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
Domain of Exposure on	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were reported on the preparation of the test media and concentrations of test substance were not measured during the study. The concentration of the solvent (acetone) was not provided.		
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. Exposures were conducted in 150mL glass flasks with HB-4 media. Temperatures and photoperiod remained consistent throughout the study, and the same algae concentration was used for all test concentrations.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 4 weeks for the chronic study.		
	Metric 11:	Number of Exposure Groups/	High	There were 5 exposure groups plus a control, and the spacing of the exposure levels was adequate for a dose response		
	Metric 12:	Testing at or Below Solubility Limit	Low	It was reported that a vehicle solvent was used, but it was unclear if the control was a solvent control. The solvent concentration was not reported.		
Domain 1: Test Organis	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The source of the S. obliquus was reported to be the Department of Pathology, the Insti- tute of Hydrobiology, Academia Sinica China.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the algae was acclimated to the test conditions prior to the start of the test		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study authors reported that a concentration of 5-7 x 10 ⁵ cells/mL of algae was used in each test chamber. The test was conducted in triplicate.		
	Continued on next page					

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Environmental Hazard Evaluation

		contin	ued from previ	ous page			
Study Citation:	Kuang, Q. J.	, Zhao, W. Y., Cheng, S. P. (2003). Toxicity of	of dibutyl phthal	ate to algae. Bulletin of Environmental Contamination and Toxicology 71(3):602-			
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:	Vegetation; 1	Non-vascular Plants; Scenedesmus obliquus;	Not Applicable	(e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	-Cell signaling/function					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1332820						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	The test was conducted at 24C with a 14L:10D photo period. The algae was cultured in 150mL flasks with HB-4 algal media.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal density-development/growth.			
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were not clearly reported but outcomes were assessed consistently across study groups. Chlorophyll a was assessed using spectrophotometry at 650nm, and cell counts were done using a hemocytometer every 2 days but methodology details were missing.			
Domain 6: Confounding	y / Variable Co	ntrol					
Domain of Comoundanty	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
		Design and Procedures		conditions. It was not reported if the algae was 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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	SGMI 1979 was used to determine EC50 values. However, it did not appear that statis- tics were performed on the other data, but independent statistical analysis may be con- ducted.			
	Metric 22:	Reporting of Data	Low	Results for the chronic exposure were reported in the text only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	This portion inclusions. I	of the evaluation is for the cellular results ittle details were provided on the chronic po	reported in the ortion of the stud	text for the chronic exposure. Cell wall thickening was reported along with cell ly. This evaluation was for the 4 week chronic exposure.			

Overall Quality Determination

Medium

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 Dibutyl phthalate (DBP) 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 or grade were not included in the technical report.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Negative controls were included in the study.		
	Metric 5:	Negative Control Response	High	There was an adequate response in the negative controls.		
	Metric 6:	Randomized Allocation	Medium	Allocation of algae for experiments was not described in the study.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The study followed OECD 201 (1981) guidelines and included any deviations.		
	Metric 8:	Consistency of Exposure	High	The exposure administration was reported as consistent.		
	Metric 9:	Measurement of Test Substance Concentration	High	Appendix A includes the analytical measurement of the chemical, gas-liquid chromatog- raphy with electron capture detection. Concentrations measured throughout the 10 days, as well as 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	An appropriate number of exposure groups was used.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Concentrations with responses were below the solubility limit.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source and details about the algae were limited.		
	Metric 14:	Acclimatization and Pretreatment	Medium	Acclimatization details were limited.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The study followed OECD 201 (1981) guidelines using adequate numbers of organisms.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome was described in detail in the results.		
Continued on next page						

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Dibutyl Phthalate

Environmental Hazard Evaluation

	continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Overall Dura Aquatic (fres uptake route) Vegetation; N Developmen Dibutyl phth 1316196	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 Dibutyl phthalate (DBP) 1316196					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were reported consistently.			
Domain 6: Confounding	y / Variable Cor Metric 19: Metric 20:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	High High	No differences were reported among the study groups. Outcomes unrelated to exposure were not reported.			
Domain 7: Data Present	ation and Anal	ysis Statistical Methods	High	Statistics used include moving average angle analysis, prohit analysis, and binomial			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	Medium High	probability. Data was reported for growth. There were no unexpected outcomes.			
Additional Comments:	None						
Overall Quality Determination		High					

Study Citation:	Adachi, A., Asa, K., Okano, T. (2006). Efficiency of rice bran for removal of di-n-butyl phthalate and its effect on the growth inhibition of Selenastrum			
Duration: Exposure Route, Media, Path:	capricornutum by di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 76(5):877-882. 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; N	Non-vascular Plants; Selenastrum capricorn	<i>utum</i> ; Not Appli	cable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Developmen	t/Growth		
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	1323217			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce	Trat Caletar a Identita	τ	
	Metric 1: Metric 2:	Test Substance Identity	LOW	The chemical was identified by name only. The source was reported as Wake Dure Chemical Industries (Amagasaki, Japan), and
	Metric 2.	Test Substance Source	Ingn	verified by gas chromatography.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design	Matria 4.	Na active Constants	TT: -1-	
	Metric 4:	Negative Control Response	High	Study authors reported using a concurrent negative control group.
	Metric 5.	Regative Control Response	Ingn	assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Demain 2. Error com Ch				
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	test concentrations.
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used. Exposures were administered consistently across study
		Administration		groups, but few details were provided.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured by gas chromatography, and given in Figure 1.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was conducted for 96 hours.
	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			
Domain 1. 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	Medium	No differences were reported in handling the algae.
	Matria 15:	Conditions	Low	
	Metric 15:	Replicates per Group	Low	An adequate number of algae were used, but replicates weren't reported.
Domain 5: Outcome Ass	sessment			
Continued on next page				

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Dibutyl Phthalate

Environmental Hazard Evaluation

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Study Citation:	Adachi, A., Asa, K., Okano, T. (2006). Efficiency of rice bran for removal of di-n-butyl phthalate and its effect on the growth inhibition of Selenastrum capricornutum by di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 76(5):877-882.				
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:	Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Development/Growth				
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	1323217				
Domain	Metric	Rating	Comments		
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance		
			of organism health but actual measured condition values for control and exposed vessels was not reported.		

	Weule 10.	Adequacy of Test Conditions	Medium	of organism health but actual measured condition values for control and exposed vessels was not reported.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but few details were 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	/ Variable Con	trol		
	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 Presenta	ation and Analy	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 Quality Determination

Medium

Study Citation:	Adachi, A., Asa, K., Okano, T. (2006). Efficiency of rice bran for removal of di-n-butyl phthalate and its effect on the growth inhibition of Selenastrum capricornutum by di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 76(5):877-882.						
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (freshwater); Water; Not determined by	y study authors (i.e., chemical of interes	t in exposure water, but unable to determine exact uptake route)				
Media, Path:							
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1323217						
Domain	Metric	Rating	Comments				

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	The source was reported as Wake Pure Chemical Industries (Amagasaki, Japan), and verified by gas chromatography.
	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 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 organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization			
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	A one-time dose was used. Exposures were administered consistently across study groups, but few details were provided.
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured by gas chromatography, and given in Figure 1.
	Metric 10:	Concentration 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	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 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	Medium	No differences were reported in handling the algae
	Metric 15:	Conditions Number of Organisms and	Low	An adequate number of algae were used, but replicates weren't reported.
		Replicates per Group		
Domain 5: Outcome As	sessment			
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported.
Continued on next page				

HERO ID: 1323217 Table: 2 of 2

continued from previous page				
Study Citation: Duration: Exposure Route, Media, Path: Taxa Species Age:	Adachi, A., Asa, K., Okano, T. (2006). Efficiency of rice bran for removal of di-n-butyl phthalate and its effect on the growth inhibition of Selenastrum capricornutum by di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 76(5):877-882. 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)			
Health Outcome:	vegetation; Non-vascular Flants; Selenastrum capricornutum; Not Applicable (e.g., lungi or algae studies) or Not Reported Development/Growth			
Chemical: HERO ID:	Dibutyl phthalate (DBP) 1323217			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but few details were 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	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, and rice bran was in all treatments.
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	ion is for the results with rice bran.		
Overall Quali	Overall Quality Determination		Medium	

Study Citation: Duration: Exposure Route	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 (freehwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route).				
Modia Dathy	Aquate (freshwater); water, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Toxo Spocios Ago:	Vegetation: 1	Non vascular Plante: Salanastrum caprico	rnutum: Not	Applicable (e.g., fungi or algae studies) or Not Peported	
Haalth Outcome	Davalanman	t/Growth	<i>mulum</i> , NOL P	Applicable (e.g., fuligi of algae studies) of Not Reported	
Chamical	Developmen Dibutyl phth	alata (DPD)			
	1321006	alate (DBI)			
	1321990				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce		Ŧ		
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported as provided by a manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.	
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.	
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	An 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 to pre- vent volatilization were not reported.	
	Metric 8:	Consistency of Exposure	High	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 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/	High	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 was performed at or below water solubility.	
Domain 4: Test Organisi	m Matri - 12	Test Organism Characteristics	T		
	Metric 13:	A colimatization of J Destructions	LOW	A source was not reported.	
	Metric 14:	Accumulization and Pretreatment	High	Appropriate acclimation 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.	

Continued on next page ...
HERO ID: 1321996 Table: 1 of 1

		conti	nued from p	revious page				
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:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratic	on: 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:	Vegetation; 1	Non-vascular Plants; Selenastrum capricor	nutum; Not A	Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
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 Outcome assessment was consistent across groups.							
		Assessment						
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant 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 Quality Determination		High						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Melin, C., E Physiologia I Overall Dura Aquatic (fres Vegetation; N Developmen Dibutyl phth 1333016	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466. 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) Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; CCAP strain 278/4; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 1333016				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control, which was a solvent control con- taining ethanol.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.		
Domain 3: Exposure Cha	aracterization Metric 7:	Experimental System/Test Media	Low	The authors provided limited details on the preparation of the DBP test solution.		
	Metric 8:	Preparation Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. Study authors reported the temperature and test volumes though.		
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors did not report if the test concentrations were measured at any point in the study.		
	Metric 10:	Exposure Duration and Frequency	Low	Study authors did not explicitly state the duration of the study, but Figure 1 indicates it was seven days.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The exposure levels were provided in Figure 1. The number of exposure groups was adequate, but different spacing (especially between 10^-5 and 10^-4M may have yielded more in-depth results.		
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent.		
Domain 4: Test Organism	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the algae was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	It was reported that the initial algal density was 0.1ug chlorophyll/mL. There were three culture flasks per concentration.		

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

			nava nom provi	here here			
Study Citation:	Melin, C., I Physiologia	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.					
Duration:	Overall Dur	ation: 4 - 10 days; Exposure Duration: 4 - 1	10 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:	Vegetation; Non-vascular Plants; Selenastrum capricornutum; CCAP strain 278/4; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1333016						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	S. capricornutum was grown in 10% (v/v) Z-8 medium according to Kotain 1972. Cultures were grown at 20 C with continuous illumination.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-algal growth.			
	Metric 18:	Consistency of Outcome Assessment	Low	It was reported that algae growth was determined by measuring changes in transmittance at 729nm, but methods and instruments used were not reported.			
Domain 6: Confoundir	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. It was not re- ported if the algae was 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	itation and Anal	lysis					
	Metric 21:	Statistical Methods	High	Growth curve determination and IC50 value determination were described in the text.			
	Metric 22:	Reporting of Data	High	Data for exposure concentrations and for the control response was reported in Figure 1 and was adequate.			

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Additional Comments: This portion of the evaluation is on the effect of DBP on the growth of algae S. capricornutum. Development/growth was selected as the outcome of interest.

Low

The study did not report any measures of variability.

Overall Quality Determination

Metric 23:

Explanation of Unexpected Outcomes

Medium

Study Citation:	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants.						
	Physiologia	Physiologia Plantarum 59(3):461-466.					
Duration: Exposure Route	Aquatic (free	ation: 0 - 4 days (0-96h); Exposure Duration shwater): Water: Not determined by study au	: 0 - 4 days (0-9 uthors (i.e., chen	00) nical of interest in exposure water, but unable to determine exact untake route)			
Media. Path:	Aquatic (IIC	require (neshwater), water, not determined by study admors (no., enemiear of interest in exposure water, but anable to determine exact aparts found)					
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Hordeum vulgare; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	Mechanistic-Photosynthesis					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1333016						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		-				
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control, which was a solvent control con- taining ethanol.			
	Metric 5:	Negative Control Response	High	The negative control response was mentioned in text under Figure 3.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the protoplasts were allocated into study groups.			
Domain 2: Exposure Ch	aractorization						
Domain 5. Exposure Cir	Metric 7.	Experimental System/Test Media	Low	The authors provided limited details on the preparation of the DRP test solution			
	Wettre 7.	Preparation	Low	The autions provided minied details on the preparation of the DDT test solution.			
	Metric 8:	Consistency of Exposure	Low	Details regarding the exposure administration were limited. Study authors reported the			
		Administration		temperature and test volumes though.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test concentrations were measured at any point in the			
		Concentration		study.			
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was described and seemed adequate.			
	Metric 11:	Number of Exposure Groups/	High	Exposure groups seemed fine and adequate results were obtained.			
	Matria 12	Spacing of Exposure Levels	High	Study authors reported the use of a valuate column			
	Metric 12:	Testing at of Below Solubility Linit	Figh	Study authors reported the use of a venicle solvent.			
Domain 4: Test Organis	m						
6	Metric 13:	Test Organism Characteristics	Low	The source of barley protoplasts was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.			
		Conditions					
	Metric 15:	Number of Organisms and	Low	The protoplast concentration was given but the number of replicates was not.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions seemed appropriate and were described on page 462 of the paper.			
		Contir	nued on next pa	nge			

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Dibutyl Phthalate

		contin	ued from previ	ous page			
Study Citation:	Melin, C., I Physiologia	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.					
Duration:	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; Hordeum vulgare; Not App	licable (e.g., fun	gi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	-Photosynthesis					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	1333016	1333016					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-photosynthesis/CO2 fixation in protoplasts from barley.			
	Metric 18:	Consistency of Outcome	High	The methodology was consistent across study groups.			
		Assessment					
Domain 6: Confounding	y / Variable Co	ntrol					
Domain 0. Combanding	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. It was not re- ported if acclimation occurred.			
	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	IC50 value determination was described in the text.			
	Metric 22:	Reporting of Data	High	Exposure data and control response were reported in Figure 3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was reported in Figure 3.			
		• • •	U				
Additional Comments:	This evaluat	ion is for the assessment of photosynthesis/O	CO2 fixation by	parley protoplasts.			
Overall Quali	ty Deterr	nination	Medium				

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Huang, Q., V Environment Overall Dura Aquatic (free Vegetation; V Mechanistic Dibutyl phth 1323213	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626. 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) Vegetation; Vascular Plants; <i>Lemna minor</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 1203213				
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	High	HPLC grade of test substance reported		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an a 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.		
Damain 2. Eastanne Ch						
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	High	Each exposures was conducted within a "glass beaker". The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	One time dose, tests repeated twice		
	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 exposure frequency were 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 suitable for a dose		
	Metric 12:	Spacing of Exposure Levels 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 adequately described and suitably conditioned to lab culture		
	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	20 fronds in triplicate is adequate		
Domain 5: Outcome As	sessment					

Continued on next page ...

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Environmental Hazard Evaluation

HERO ID: 1323213 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation:	Huang, Q., V Environment	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health Part A: Toxic/Hazardous Substances & Environmental Environmental Environmental Science and Health Part A: Toxic/Hazardous Substances & Environmental Environmental Environmental Science and Health Part A: Toxic/Hazardous Substances & Environmental Environmental Environmental Science and Health Part A: Toxic/Hazardous Substances & Environmental Envinto Environmental Envitor Environmental Env					
Duration:	Overall Dura	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:	-						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Lemna minor; Not Applica	able (e.g., fur	igi or algae studies) or Not Reported			
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cell sig	naling/function	on-Oxidative stress (including redox biology)-Photosynthesis			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323213						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not 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	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	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:	ts: Subtitles of Figure 1 and 2 were apparently switched. This form represents the mechanistic endpoints from the paper and include: Cell signaling/function- Oxidative stress (including redox biology)-Photosynthesis for Lemna						
Overall Qualit	ty Detern	nination	High				

Study Citation:	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of					
Duration	Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626.					
Duranon; Evnosure Route	Aquatic (free	when the study of	10 uays	chemical of interest in exposure water, but unable to determine exact untake route)		
Media Path.	Aquatic (fies	sinvater), water, not determined by study	autions (1.e., 6	enclinear of interest in exposure water, but unable to determine exact uptake route)		
Taxa Snecies Age	Vegetation: Vascular Plants: Lemna minor: Not Applicable (e.g. fungi or algae studies) or Not Reported					
Health Outcome	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	1323213					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only and purchased from Sigma.		
	Metric 2:	Test Substance Source	Low	"DBP (HPLC grade) was purchased from Sigma (USA)."		
	Metric 3:	Test Substance Purity	High	The test substance was reported to be HPLC grade.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using a 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	Hıgh	Each exposure was conducted within a "glass beaker". The experimental system and methods for preparation of test media were described in adequate detail		
	Metric 8:	Consistency of Exposure	High	A one-time dose was used. Tests were repeated twice.		
		Administration	i iigii			
	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	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 ($<7.5 \text{ mg/L}$) were below the water solubility limit (11.2 mg/L).		
		· · ·	-			
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	High	The test organisms were collected from Tai Lake in China, were adequately described and suitably conditioned to lab culture.		
	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	Twenty fronds in triplicate is adequate.		
		Replicates per Group				
Domain 5. Outcome A-	ansmart					
Domain 5: Outcome Ass	Matria 16	A dequeey of Test Conditions	Madium	Environmental conditions of the test system were most likely conducive to maintenence		
	Metric 10:	Adequacy of rest Conditions	wiedium	of organism health but actual measured condition values for control and exposed vessels was not reported.		
		Cont	inued on nex	t page		

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Environmental Hazard Evaluation

HERO ID: 1323213 Table: 2 of 2

		conti	nued from p	previous page			
Study Citation:	Huang, Q., V	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of					
	Environmen	Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	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:	Vegetation;	Vascular Plants; <i>Lemna minor</i> ; Not Applica	able (e.g., fur	ngi or algae studies) or Not Reported			
Health Outcome:	Developmen	tt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323213						
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 Co	ntrol					
Domain 0. Comountaing	Matria 10:	Confounding Variables in Test	Low				
	Metric 19:	Design and Designations	Low	conditions			
	Matria 20.	Design and Procedures	Madium	There was no information in the study to suggest differences among groups			
	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	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described. Treatments were compared to the control using Student's t-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:	The subtitles	s of Figures 1 and 2 were apparently switch	ed.				
Overall Quali	ty Deterr	nination	High				

Overall Quality Determination

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Melin, C., F Physiologia Overall Dura Aquatic (free Vegetation; ^V Mechanistic Dibutyl phth 1333016	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466. 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>Spinacea oleraceae</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Photosynthesis Dibutyl phthalate (DBP) 1333016					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control, which was a solvent control con-			
	Matria 5.	Negeting Control Decomposition	TT: -1-	taining ethanol.			
	Metric 5:	Dendemined Allegation	High	The negative control response was mentioned in the text under Figure 4.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the thylakoids were allocated into study groups.			
Domain 3: Exposure Ch	aracterization	Europius antal Cardona (Erad Madia	T and				
	Metric 7:	Preparation	Low	The authors provided limited details on the preparation of the DBP test solution.			
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. Study authors reported the temperature and test volumes though.			
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors did not report if the test concentrations were measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was described and seemed adequate.			
	Metric 11:	Number of Exposure Groups/	High	Exposure groups seemed fine and adequate results were obtained.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent.			
Domain 4: Test Organis	m						
8	Metric 13:	Test Organism Characteristics	Low	The source of spinach thylakoids was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The thylakoid concentration and number of replicates was not reported.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions seemed appropriate and were described on page 462 of the paper.			
Continued on next page							

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 1333016 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation:	Melin, C., I Physiologia	Melin, C., Egneus, H. (1983). Effects of di-n-butyl phthalate on growth and photosynthesis in algae and on isolated organelles from higher plants. Physiologia Plantarum 59(3):461-466.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) A quatic (freehyster). Weter: Net determined by study outbars (i.e., shernical of interact in exposure yeter, but yeahls to determine exact yeters are to be a set of the set of						
Exposure Koute, Modia Datha	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)					
Toyo Species Ages	Vagatation: Vagaular Dlants: Sningagg alargagge: Not Applicable (2.5. function alogo studios) or Not Deported						
Hoolth Outcomo:	Mechanistic	Vegetation; Vascular Plants; Spinacea oleraceae; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Chamical:	Dibutyl phth	-1 hotosynthesis					
HFRO ID:	1333016	lalate (DBI)					
Domain	1555010	Metric	Rating	Comments			
Domain	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–electron transport reactions in thylakoids from spinach.			
	Metric 18:	Consistency of Outcome	High	The methodology was consistent across study groups.			
		Assessment	-				
Domain 6: Confounding	o / Variable Co	ntrol					
2011111 01 0011041141	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. It was not reported if acclimation occurred			
	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					
Domain 7. Dua Presen	Metric 21	Statistical Methods	High	IC50 value determination was described in the text			
	Metric 22:	Reporting of Data	High	Exposure data and control response were reported in Figure 4			
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was reported in Figure 4.			
		1	0	, Internet Only and			
Additional Comments:	This evaluat	ion is for the assessment of photosynthesis/e	electron transfer	reactions by spinach thylakoids.			
Overall Quali	ty Deterr	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Huang, Q., V Environment Overall Dura Aquatic (free Vegetation; ^v Mechanistic Dibutyl phth 1323213	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 41(8):1615-1626. 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) Vegetation; Vascular Plants; <i>Spirodela polyrhiza</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 1323213				
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	High	HPLC grade of test substance reported		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using an a 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	High	Each exposures was conducted within a "glass beaker". The experimental system and methods for preparation of test media were described in adequate detail.		
	Metric 8:	Consistency of Exposure	High	One time dose, tests repeated twice		
	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 exposure frequency were 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 suitable for a dose response		
	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 adequately described and suitably conditioned to lab culture		
	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	20 fronds in triplicate is adequate		
Domain 5: Outcome Ass	sessment					

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 1323213 Table: 1 of 2

		conti	nued from p	revious page		
Study Citation:	Huang, Q., V Environment	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of Environmental Science and Health. Part A: Toxic/Hazardous Substances & Environmental Environmental Environmental Science and Health.				
Duration:	Overall Dura	Dverall 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:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Spirodela polyrhiza; Not A	Applicable (e.	g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-	Biomarkers (exposure and effect)-Cell sig	naling/functi	on-Oxidative stress (including redox biology)-Photosynthesis		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323213					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not 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		
Domain 6: Confounding	y / Variable Cou	Assessment				
Domain 0. Comounding	Metric 19.	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	meure 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		
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:	subtitles of Figure 1 and 2 were apparently switched. This form represents the mechanistic endpoints from the paper and include: Cell signaling/function- Oxidative stress (including redox biology)-Photosynthesis for Spirodela polyrhiza					
Overall Qualit	ty Detern	nination	High			

Study Citation:	Huang, Q., Wang, Q., Tan, W., Song, G., Lu, G., Li, F. (2006). Biochemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of						
Duration	Overall Dura	al Science and Health, Part A: Ioxic/Haza	irdous Substa 10 days	nces & Environmental Engineering 41(8):1615-1626.			
Exposure Route.	Aquatic (fres	shwater): Water: Not determined by study:	authors (i.e.)	chemical of interest in exposure water, but unable to determine exact untake route)			
Media. Path:	riquare (irea	invatory, valor, rot determined by study	uuiiois (i.e., ·				
Taxa, Species, Age:	Vegetation: V	Vascular Plants: <i>Spirodela polyrhiza</i> : Not A	Applicable (e.	g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth	ipplicacie (ei	g, range of algae staates) of not reported			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323213						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only and purchased from Sigma.			
	Metric 2:	Test Substance Source	Low	"DBP (HPLC grade) was purchased from Sigma (USA)."			
	Metric 3:	Test Substance Purity	High	HPLC grade of test substance was reported.			
Domain 2: Test Design							
-	Metric 4:	Negative Controls	High	Study authors reported using a negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for			
	Metric 6	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups			
	Medie 0.	Randoniized Anocation	Low	Researchers and not report now organisms were anotated to study groups.			
Domain 3: Exposure Ch	aracterization						
1	Metric 7:	Experimental System/Test Media	High	Each exposure was conducted within a "glass beaker". The experimental system and			
		Preparation	e	methods for preparation of test media were described in adequate detail.			
	Metric 8:	Consistency of Exposure	High	A one-time dose was used. Tests were repeated twice.			
	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 exposure frequency were 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 suitable for a dose			
	Matria 12.	Spacing of Exposure Levels	High	Exposure concentrations ($< 7.5 \text{ mg/l}$) were below the water solubility limit (11.2 mg/l)			
	Wieute 12.	Testing at of Below Solubility Ellint	Ingn	Exposure concentrations (< 7.5 mg/L) were below the water solubility mint (11.2 mg/L).			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	High	The test organisms were collected from Tai Lake in China, adequately described and suitably conditioned to lab culture.			
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and	Medium	Twenty fronds in triplicate is adequate.			
		Replicates per Group					
Domain 5: Outcome Ass	Demain & Orderman Assessment						
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health, but actual measured condition values for control and exposed vessels was not reported.			
		Cont	inued on nex	at page			

Environmental Hazard Evaluation

HERO ID: 1323213 Table: 2 of 2

		conti	nued from p	revious page			
Study Citation:	Huang, Q., V	Wang, Q., Tan, W., Song, G., Lu, G., Li, F.	(2006). Bio	chemical responses of two typical duckweeds exposed to dibutyl phthalate. Journal of			
	Environmen	tal Science and Health, Part A: Toxic/Haza	rdous Substa	nces & Environmental Engineering 41(8):1615-1626.			
Duration:	Overall Dura	Overall Duration: 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:	Vegetation; '	Vascular Plants; Spirodela polyrhiza; Not A	Applicable (e.	g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323213						
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	v / Variable Co	ntrol					
Domain 0. Comountaing	Matria 10:	Confounding Variables in Test	Low				
	Metric 19:	Design and Deservices in Test	Low	conditions			
	Matria 20:	Outcomes Unrelated to Exposure	Madium	There was no information in the study to suggest differences among groups			
	Mettic 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	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described. Treatments were compared to the control using Student's t-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:	The subtitles	s of Figures 1 and 2 were apparently switch	ied.				
Overall Qualit	ty Detern	nination	High				

Overall Quality Determination

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: Exposure Route, Media, Path:	Overall Dura Aquatic (fres	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:	Vegetation; V Reproductive Dibutyl phth 3515118	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 3515118						
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	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	The 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 Cha	aracterization							
	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.				
	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 suitable for a dose				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	response. 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 Oreania								
Domain 4: Test Organisi	II Matria 12:	Test Organism Characteristic-	High					
	Matria 14:	A colimatization and Protrostroant	High	I ne test organisms were adequately described and were obtained from a reliable source.				
	Metric 14:	Conditions	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 Assessment

Continued on next page ...

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Dibutyl Phthalate

continued from previous 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: 0 - 4 days (0-96h); Exposure Durati	on: 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; V	Vascular Plants; Triticum sp.; Jinnong 7; N	lot Applicable	e (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3515118						
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 germination experiment was conducted in a "growth chamber in total darkness at a temperature of 25 ± 10 C 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 were 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 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 Present	ation and Anal	ysis					
		~ · · · · ·					

Domain /: Data Presenta	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). IC10 and IC50 values were provided for root and shoot elongation.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	This evaluation form is relevant for germination rate and shoot/root growth.						

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media Path:	Gao, M., Do (2-ethylhexy Overall Dura Aquatic (fres	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)				
Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; V ADME (biot Dibutyl phth	/ascular Plants; <i>Triticum sp</i> .; Jinnong 7; N ransformation) alate (DBP)	ot Applicable	e (e.g., fungi or algae studies) or Not Reported		
Domain	3515118	Matric	Pating	Comments		
Domain 1: Test Substan	re .	Metric	Katilig	Comments		
Domain 1. Test Substant	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS		
	Metric 2:	Test Substance Source	Low	Source 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 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	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 9: Exposure on	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were adequately reported. 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 spacing of exposure levels were suitable for a dose response		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration was reported but the biological response of the control was not.		
Domain 4: Test Organisi	m					
station of games	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 Assessment						
Continued on next page						

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 3515118 Table: 1 of 3

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Gao, M., Do (2-ethylhexy Overall Dura Aquatic (free Vegetation; ADME (biot Dibutyl phth 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: 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 ADME (biotransformation) Dibutyl phthalate (DBP) 3515118				
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 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".		
	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 (percent recovery, method detection limit, etc.) for HLPC analysis were not provided.		
	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	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	ysis				
	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. Control response was not provided.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Control grou	p was not analyzed for DBP. The exposure	concentratio	ons were not verified.		
Overall Qualit	ty Deterr	nination	High			

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HERO ID: 3515118 Table: 2 of 3

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	Diverall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Vegetation;	Vascular Plants; Triticum sp.; Jinnong 7; N	ot Applicabl	e (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)-Oxidati	ve stress (inc	eluding redox biology)					
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	3515118								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS					
	Metric 2:	Test Substance Source	Low	Source 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 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 Characterization

The experimental system and/or test media preparation methods were adequately reported. The test solutions were replenished daily. Exposures were administered consistently across study groups. Exposure concentrations were not measured.
Exposure concentrations were not measured.
Exposures were administered consistently across study groups. Exposure concentrations were not measured.
Exposure concentrations were not measured.
The duration of exposure was reported and appropriate for the study type
The number of exposure groups and spacing of exposure levels were suitable for a dose
response
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.
1

Domain 4: Test Organism

Conditions Metric 15: 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	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 to character- ize toxicological effects

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 3515118 Table: 2 of 3

		conti	nued from p	revious page				
Study Citation:	Gao, M., Do (2-ethylhexy	ong, Y., Zhang, Z., Song, W., Qi, Y. (201 1) phthalate stress. Chemosphere 172(Elsev	7). Growth vier):418-428	and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di				
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:	Mechanistic	Biomarkers (exposure and effect)-Oxidativ	ve stress (inc	luding redox biology)				
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	3515118							
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 performed in an artificial climate chamber with alternating temperatures of 25 ± 1 oC (12 h light) and 20 ± 1 oC (12 h dark), 60% relativehumidity, and a light intensity of 40 uM/ms".				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodologies for enzyme activities were not clearly reported.				
	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	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 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 form is relevant to all mechanistic endpoints including antioxidant enzyme activities, lipid peroxidation, O2 accumulation and plasma membrane permeability.							
Overall Quali	ty Detern	nination	High					

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HERO ID: 3515118 Table: 3 of 3

Study Citation:	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 athulhayul) phthalate atroac. Chamcaphare 172(Elequicre):418, 428					
Duration:	Overall Duration: 11 - 21 days; Exposure Du	iration: 11 - 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:	Vegetation; Vascular Plants; Triticum sp.; Jin	nong 7; Not Applicable (e.g., fungi or	algae studies) or Not Reported			
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	3515118					
Domain	Metric	Rating	Comments			

Domain		Wieuric	Kating	Comments	
Domain 1: Test Substan	ice				
	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					
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	Passarchers did not report how organisms were allocated to study groups	
	Wietrie 0.	Kandoniized Anocation	LOW	Researchers and not report now organisms were anocated to study groups.	
Domain 3: Exposure Ch	naracterization				
	Metric 7:	Experimental System/Test Media	Medium	The experimental system and test media preparation methods were adequately reported.	
		Preparation		The test solutions were replenished daily.	
	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:	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 suitable for a dose	
		Spacing of Exposure Levels	8	response.	
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration but the bio-	
		5		logical response of the solvent control was acceptable and no interactions are expected	
				between the solvent and test substance.	
Domain 4 [.] Test Organis	m				
Domain 1. 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	
	Medic 11.	Conditions	Ingn	The prededucinent conditions were the same for condition and exposed organisms.	
	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				
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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 3515118 Table: 3 of 3

		conti	nued from p	revious page				
Study Citation: Duration:	Gao, M., Do (2-ethylhexy Overall Dura	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						
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:	T T							
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Triticum sp.</i> ; Jinnong /; No	ot Applicable	e (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	alata (DBD)						
HERO ID:	3515118	alate (DBF)						
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 performed in an artificial climate chamber with alternating temperatures of 25 ± 1 oC (12 h light) and 20 ± 1 oC (12 h dark), 60% relative 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 surfacearea, 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 Confounding	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 (e.g., infection) 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 (Table 2 and Fig 3).				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	Root morph exposure con	ology measurements included total root le ncentrations were not verified.	ength, total r	oot surface area, average root diameter, and the number of root tips and hairs. The				
Overall Quali	ty Detern	nination	High					

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Liang, W., Deng, J. Q., Zhan, F. C., Wu, Z. B. (2009). Effects of constructed wetland system on the removal of dibutyl phthalate (DBP). Microbiological						
Duration	Research 164	Research $164(2):206-211$.					
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:	riquine (ires						
Taxa, Species, Age:	Other; Fungu	s; fungus; Not Applicable (e.g., fungi or alg	ae studies) or Not Report	ed			
Health Outcome:	Development	/Growth					
Chemical:	Dibutyl phtha	alate (DBP)					
HERO ID:	1323196						
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	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							
8	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control system.			
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes. There was no report of initial population numbers.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Char	racterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided no details on the measures taken to appropriately prepare test con- centrations.			
	Metric 8:	Consistency of Exposure	Low	It is unclear when and how DBP was added to the system.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and appropriate for the study type, but it is un- clear when dosing occurred.			
	Metric 11:	Number of Exposure Groups/	Low	Two dissimilar doses were applied at each phase, but the phase was not defined.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.			
Domain 4. Test Organism							
Domain 1. 10st Organishi	Metric 13:	Test Organism Characteristics	Uninformative	Test organisms were not added to the system and the source was not reported			
	Metric 14:	Acclimatization and Pretreatment	N/A	Organisms may have been previously exposed to the test substance or other unintended			
		Conditions		stressors.			
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	The initial number of organisms was not reported.			
Domain 5: Outcome Asse	ssment						
2 smail 5. Sucome Asso	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		С	ontinued on next page				

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Environmental Hazard Evaluation

HERO ID: 1323196 Table: 1 of 1

		col	ntinued from previous	s page		
Study Citation:	Liang, W., D	Liang, W., Deng, J. Q., Zhan, F. C., Wu, Z. B. (2009). Effects of constructed wetland system on the removal of dibutyl phthalate (DBP). Microbiological				
Duration:	Overall Dura	4(2).200-211.	IVS			
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:	1	····· , ··· , ··				
Taxa, Species, Age:	Other; Fung	us; <i>fungus</i> ; Not Applicable (e.g., fungi or alga	e studies) or Not Repor	rted		
Health Outcome:	Developmen	t/Growth	•			
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323196					
Domain	Metric Rating Comments					
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes of interest.		
	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 Co	ntrol				
	Metric 10	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.		
	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	N/A	Statistical analysis was not possible (n=1).		
	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:	Enzymes we	ere also measured but not attributed to any enti-	ity.			

Overall Quality Determination

Uninformative

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 Dibutyl phthalate (DBP) 1316224					
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 test material was identified and a 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. Purity and/or grade of the test substance were not reported		
		Test Substance Fully	Low	r any and of 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	High	The experimental system and methods for preparation of test media were described in adequate detail and accounted for the properties of the test material. For all low- solubility phthalates, enhanced mixing procedure was used, while for butyl benzyl ph- thalate exposure microbial degradation was accounted for with a cleaning procedure 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 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:	Exposure Duration and Frequency	High	The 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					
-	Metric 13:	Test Organism Characteristics	High	Specimens were either cultured at the Laboratory orpurchased commercially. All fish were tested as juveniles at <10weeks old.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	A 96-hour acclimation period was reported.		
Continued on next page						

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Dibutyl Phthalate

... continued from previous page **Study Citation:** Bionomics, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report). **Duration:** 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 **Exposure Route**, Media, Path: uptake route) Taxa, Species, Age: Vertebrate; Fish; sheepshead minnow (Cyprinodon variegatus); Juvenile **Health Outcome:** Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 1316224 Domain Metric Rating Comments Number of Organisms and Metric 15: Low Only two replicates of 10 fish were used in each treatment. Replicates per Group Domain 5: Outcome Assessment Adequacy of Test Conditions Metric 16: High Organism housing, environmental conditions, food, water, and nutrients were conducive to 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 High Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Medium The study reported minor differences among the study groups with respect to environmental conditions or other non-treatment-related factors, but these are unlikely to Design and Procedures have a substantial impact on results. Authors reported that dissolved oxygen fell below guideline 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., 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 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 explained. Low DO did not have an affect on the outcome and high mortality in controls was not reported for this chemical. Additional Comments: Study report for the Sheepshead minnow test begins on pg 124 of the PDF. DEHP is referred to as phthalate 1H, Dibutyl Phthalate was referred to as 1C,

and Butyl Benzyl Phthalate was referred to as 1D.

Overall Quality Determination	High

	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.						
Duration: Exposure Route, Media Path:	Overall Dura Aquatic (mar	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome:	Invertebrate; Development	Other Invertebrate (e.g., sea urchins, ciliate t/Growth	s, rotifers); Anin	nalia; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Chemical: HERO ID:	Dibutyl phth 5495608	alate (DBP)					
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	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was 100%.			
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 initial placement of aquaria was determined.			
Domain 2: Exposure Ch	araatarization						
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 there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it was assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.			
		Contin	nued on next pa	ge			

HERO ID: 5495608 Table: 1 of 2

		contin	ued from previ	ous page			
Study Citation:	Tagatz, M. E	Fagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration:	Overall Dura	Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., chemica	l 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); Anin	nalia; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
		Design and Procedures	C				
	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	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:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic							
	communities	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.						
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate;	Other Invertebrate (e.g., sea urchins, ciliate	es, rotifers); Anin	<i>ualia</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5495608							
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	The test substance was identified using electron-capture gas-liquid chromatography.				
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was 100%.				
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 initial placement of aquaria was determined.				
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 there is some concern over the use of plastic trays.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.				
	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	8	response.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.				
Domain 4: Test Organis	III Matria 12:	Test Organism Characteristics	Madium	Naturally adaptizing organisms ware used, and it is assumed there was equal initial				
	Metric 15.	Test Organism Characteristics	Medium	population structure.				
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-				
	M (15	Conditions	т	18ms.				
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms 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 adequate.				
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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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Dibutyl Phthalate

		contir	ued from previo	ous page		
Study Citation:	Tagatz, M. E	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic				
Duration:	Overall Dura	ation: 11 - 21 days: Exposure Duration: 11 -	· 21 days	(5).259-240.		
Exposure Route.	Aquatic (ma	rine): Water: Not determined by study author	ors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)		
Media. Path:	i iquaite (iiia		, ••			
Taxa. Species. Age:	Invertebrate:	Other Invertebrate (e.g., sea urchins, ciliate	s. rotifers): Anim	alia: Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth	-,,,,	, · · · · · · · · · · · · · · · · ·		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5495608					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	y / Variable Co	ntrol				
Domain 0. Comounding	Metric 19	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
	metile 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 was no information in the study to suggest differences among groups.		
Domain 7: Data Present	ation and Anal	veic				
Domain 7. Data Fresent	Motrio 21:	ysis Statistical Methods	High	Statistical methods were adaquately described		
	Metric 22:	Peporting of Data	High	Date for exposure related findings were presented for each treatment and control group		
	Metric 22.	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained		
	Wiettie 25.	Explanation of Onexpected Outcomes	Ingn	Onexpected outcomes were satisfactority explained.		
Additional Comments:	None					
Overall Quality Determination Medium						

Study Citation:	Tagatz, M. E	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (ma	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 5495608	Worms (e.g., Annelids, Nematodes); Annel t/Growth alate (DBP)	<i>lida</i> ; multiple spe	ecies; Not Applicable (e.g., fungi or algae studies) or Not Reported			
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	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
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 initial placement of aquaria was determined.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately re- ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered 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	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.			
		Conti	nued on next pa	ge			

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continued from previous page						
Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5495608					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 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	vsis				
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 22:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained		
	metric 25.	Explanation of Chexpected Outcomes	ingn	chexpected outcomes were substactority explained.		
Additional Comments:	None					
Overall Qualit	ty Deterr	nination	Medium			

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Durations	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration: Evnosuro Douto	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days A quetic (marine). Weter: Not determined by study outbors (i.e., shemical of interact in supersure water, but unable to determine sweet untake route).					
Exposure Route, Modia Dathy	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Tava Spacios Ago:	Investabusta Warma (a.a. Annalida Namatadas), Annalida multipla anagian Nat Annligabla (a.a. fungi analaga studica) an Nat Danastad					
Hoolth Outcome	Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Chamical:	Development/Growth Dibutyl phthelate (DBD)					
HERO ID:	5495608					
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	The test substance was identified using electron-capture gas-liquid chromatography.		
	Metric 3:	Test Substance Purity	Low	The purity of test substance was 100%.		
Domain 2: Test Design						
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		
	Weate 5.	reguive control response	mgn	assessed outcomes.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-		
	Wietrie 7.	Preparation	Wiedrum	ported but there is some concern over the use of plastic trays.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9.	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies		
	Methe 9.	Concentration	mgn	Exposure concentrations were measured using appropriate analytical commongles.		
	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		
	Matria 12	Spacing of Exposure Levels	Uiah	response.		
	Metric 12:	Testing at of Below Solubility Limit	Figh	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organis	m					
C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial		
	Metric 14.	Acclimatization and Pretreatment	Medium	population structure. All pretreatment conditions were most likely the same for control and exposed organ-		
	Methe 11.	Conditions	Wiedium	isms.		
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.		
		Replicates per Group				
Domain 5: Outcome Ass	sessment					
2 smain 5. Outcome / Is	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 (harvesting) 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.		
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May 2025 Environmental Hazard Evaluation

HERO ID: 5495608 Table: 2 of 2

			ava nom provi	ous page		
Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5495608					
Domain		Metric	Rating	Comments		
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		
	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 Presenta	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.		

Study Citation:	Sugawara, N. (1974). Effect of phthalate esters on shrimp. Bulletin of Environmental Contamination and Toxicology 12(4):421-424.						
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratior	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;	Arthropods; Artemia salina; Larvae					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5569571						
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	nce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design			··· ·				
	Metric 4:	Negative Controls	High	A concurrent negative control was used in the study.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and was adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the brine shrimp larvae were allocated into study groups.			
Domain 3: Exposure Cl	naracterization		T				
	Metric /:	Experimental System/Test Media Preparation	Low	Petri dishes were used as the test chambers, but little information was provided on the preparation of the test substance.			
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. All exposures were for 24h in 9 x 2cm petri dishes, but more information was needed.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24h. This was adequate to determine the hatch.			
	Metric 11:	Number of Exposure Groups/	Medium	There were only three exposure levels, but the spacing was adequate to see a response.			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	The highest two concentrations exceeded the water solubility limit, and it was not re- ported if a vehicle solvent was used.			
Domain 4: Test Organis	Metric 12.	Test Organism Characteristics	Low	The course of the shrimp was only reported to be a commercial source. More informed			
	Metric 15.	Test Organism Characteristics	Low	tion is necessary.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Number of Organisms and	Medium	There were 10 larvae in each petri dish with 3-6 replicates per exposure level.			
		Replicates per Group					
Domain 5: Outcome As	Domain 5: Outcome Assessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Tests were carried out at 26C, but little other information on the environmental condi-			
				tions was reported.			
Continued on next page							

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Dibutyl Phthalate

... continued from previous page Sugawara, N. (1974). Effect of phthalate esters on shrimp. Bulletin of Environmental Contamination and Toxicology 12(4):421-424. **Study Citation:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Duration:** 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; Artemia salina; Larvae **Health Outcome:** Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 5569571 Domain Metric Rating Comments Metric 17: Outcome Assessment Methodology High The outcome assessment methodology addressed or reported the intended outcome of interest-larvae survival. Metric 18: Consistency of Outcome High After 24h, the number of larvae mortalities was counted. This was determined by whether the larvae moved or not. 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 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 Low Statistical analysis was performed but not described. Metric 22: Reporting of Data High Data for each exposure response and the control response was reported in Table 1 and was adequate for the outcome of interest. 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 of the evaluation was on the effect of DBP on the survival of A. salina larvae. Mortality was selected as the outcome of interest. The study received an unacceptable ranking due to the two highest test concentrations being above the the water solubility limit and the lack of reporting on a vehicle solvent

Overall Quality Determination

Uninformative

HERO ID: 5569571 Table: 2 of 2

Study Citation: Duration: Exposure Route, Media Path:	Sugawara, N. (1974). Effect of phthalate esters on shrimp. Bulletin of Environmental Contamination and Toxicology 12(4):421-424. 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 5569571	Invertebrate; Arthropods; <i>Artemia salina</i> ; Embryo Mortality Dibutyl phthalate (DBP) 5569571				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		Ŧ			
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source of DBP was not reported.		
	Metric 5:	Test Substance Fullty	Low	The purity/ grade of the DBP was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	A concurrent negative control was used in the study.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the brine shrimp embryos were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	Petri dishes were used as the test chambers, but little information was provided on the preparation of the test substance.		
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. All exposures were for 24h in 9 x 2cm petri dishes, but more information was needed.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24h. This was adequate to determine the hatch.		
	Metric 11:	Number of Exposure Groups/	Medium	There were only 3 exposure levels, but the spacing was adequate to see a response.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Uninformative	The highest 2 concentrations exceeded the water solubility limit, and it was not reported if a vehicle solvent was used.		
Domain 4: Test Organis	m					
C	Metric 13:	Test Organism Characteristics	Low	The source of the shrimp was only reported to be a commercial source. More informa- tion is necessary.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 1-2g of embryos in each petri dish. The number of replicates was not reported.		
Domain 5: Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Tests were carried out at 26C, but little other information on the environmental condi- tions was reported		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–the number of embryos that hatched.		
		С	Continued on next page	•		

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		cont	tinued from previou	s page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Sugawara, N Overall Dura Aquatic (ma Invertebrate: Mortality Dibutyl phth	Sugawara, N. (1974). Effect of phthalate esters on shrimp. Bulletin of Environmental Contamination and Toxicology 12(4):421-424. 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>Artemia salina</i> ; Embryo Mortality Dibutyl phthalate (DBP)				
Domain	5569571	Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	Low	After 24h, the number of nauplius was counted. More details are needed on the assess- ment procedure to determine consistency.		
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	lysis				
	Metric 21:	Statistical Methods	High	Data was analyzed according to the t-test.		
	Metric 22:	Reporting of Data	High	Data for each exposure response and the control response was reported in Figure 1 and was adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 1.		
Additional Comments:	This portion of the evaluation was on the effect of DBP on the hatch of A. salina embryos. Mortality was selected as the outcome of interest. The study received an unacceptable ranking due to the two highest test concentrations being above the the water solubility limit and the lack of reporting on a vehicle solvent					
Overall Qualit	ty Deterr	nination U	U ninformativ	ve		

Study Citation: Duration:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days							
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study authors	ors (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taya Species Age:	Invertebrate: Arthropods: Arthropods: Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Invertebrate; Arthropods; Arthropoda; Not Applicable (e.g., fungi of algae studies) or Not Reported Development/Growth							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	5495608	495608						
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	The test substance was identified using electron-capture gas-liquid chromatography.				
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.				
Domain 2: Test Design								
C C	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 initial placement of aquaria was determined.				
	, . ,.							
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Madium					
	Metric 7:	Preparation	Medium	ported but there is some concern over the use of plastic trays.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.				
	Metric 10:	Concentration 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	e	response.				
	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	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.				
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.				
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 adequate.				
		Conti	nued on next pa	ge				

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Environmental Hazard Evaluation

HERO ID: 5495608 Table: 1 of 2

continued from previous page					
Study Citation:	Tagatz, M. H	E., Deans, C. H., Moore, J. C., Plaia, G. R.	(1983). Alteration	as in composition of field-developed and laboratory-developed estuarine benthic	
	communities	s exposed to di-normal-butyl phthalate. Aqu	atic Toxicology 3	(3):239-248.	
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (ma	rine); 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:	Invertebrate	; Arthropods; Arthropoda; Not Applicable (e.g., fungi or algae	e studies) or Not Reported	
Health Outcome:	Developmen	nt/Growth			
Chemical:	Dibutyl phth	nalate (DBP)			
HERO ID:	5495608				
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 & Confounding	Variable Ca	ntrol			
Domain 0. Comountaing	Matria 10:	Confounding Variables in Test	High	There were no reported differences among the study around in environmental conditions	
	Wieute 19.	Design and Procedures	mgn	There were no reported unreferences 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	lysis			
	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:	None				
Overall Quality Determination		Medium			

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.						
Duration: Exposure Route, Media, Path:	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact up						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 5495608	nvertebrate; Arthropods; <i>Arthropoda</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5495608					
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	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
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 initial placement of aquaria was determined.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-			
	Wette 7.	Preparation	Wiedrum	ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered 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	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						
C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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 (harvesting) 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.			
	Continued on next page						

continued from previous page						
Study Citation:	Tagatz, M. E communities	Fagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.				
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study autho	ors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Arthropoda; Not Applicable (e	e.g., fungi or alga	e studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5495608					
Domain		Metric Rating Comments				
Domain 6: Confounding	v / Variabla Co	ntrol				
Domain 0. Comountaing	Metric 10	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions		
	Wieure 19.	Design and Procedures	Ingn	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				
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	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	None					
Overall Quality Determination Medium						

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic						
Duration: Exposure Route, Media, Path:	communities Overall Dura Aquatic (ma	Sommunities exposed to di-normal-bury phinaitate. Aquatic Toxicology 5(5):259-248. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Chordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not						
	Reported						
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
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	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
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 initial placement of aquaria was determined.			
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 there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration 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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response. Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organis	m						
-	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.			
		Conti	nued on next pa	ge			
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HERO ID: 5495608 Table: 1 of 2

		conti	nued from previo	bus page			
Study Citation:	Tagatz, M. E	E., Deans, C. H., Moore, J. C., Plaia, G. R.	(1983). Alteratio	ns in composition of field-developed and laboratory-developed estuarine benthic			
Duration:	Overall Dur	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	require (marme), when, not determined by study address (ne., enclined of interest in exposure water, but and be to determine exact aptake route)						
Taxa, Species, Age:	Invertebrate	Other Invertebrate (e.g., sea urchins, cili	ates, rotifers); Cl	hordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not			
	Reported						
Health Outcome:	Developmen	nt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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	y / 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	Medium	There was no information in the study to suggest differences among groups.			
Domain 7: Data Present	tation and Anal	lysis					
	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:	None						
Overall Quali	ty Deterr	nination	Medium				

Study Citation:	Tagatz, M. H	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (ma	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Chordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not					
Health Outcome: Chemical: HERO ID:	Reported Developmen Dibutyl phth 5495608	Reported Development/Growth Dibutyl phthalate (DBP) 5495608					
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	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2. Test Design							
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 initial placement of aquaria was determined.			
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 there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration 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	U	response.			
	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	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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 (harvesting) was not clearly reported.			
Continued on next page							

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HERO ID: 5495608 Table: 2 of 2

		contin	ued from previo	us page			
Study Citation:	Tagatz, M. E communities	Fagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Other Invertebrate (e.g., sea urchins, cilia	ates, rotifers); Ch	uordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not			
	Reported						
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Low	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	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	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:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.							
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (mai	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Development Dibutyl phthe 5495608	nvertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Coelenterata</i> ; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5495608						
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	High	The test substance was identified using electron-capture gas-liquid chromatography.				
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.				
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 initial placement of aquaria was determined.				
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 there is some concern over the use of plastic trays.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.				
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit.				
Domain 4: Test Organis	m							
C C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.				
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.				
		Contin	nued on next pa	ge				

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HERO ID: 5495608 Table: 1 of 2

		contir	ued from previ	ous page			
Study Citation:	Tagatz, M. H	E., Deans, C. H., Moore, J. C., Plaia, G. R.	(1983). Alteratio	ons in composition of field-developed and laboratory-developed estuarine benthic			
	communities	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., chemica	l 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	es, rotifers); Coel	enterata; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	nt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 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	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	Unavascial outcomes were satisfactorily evaluated			
	Metric 23.	Explanation of Onexpected Outcomes	Ingh	Unexpected outcomes were satisfactority explained.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration: Exposure Doute	Aquatic (ma	ation: 11 - 21 days; Exposure Duration: 11 -	· 21 uays	Lof interact in avacuura water, but unable to determine evact untake route)		
Media Path.	Aquatic (IIIa	inne), water, not determined by study autic	JIS (I.e., chennea	i of interest in exposure water, but unable to determine exact uptake route)		
Taxa Snecies Age	Invertebrate	Other Invertebrate (e.g. sea urchins, ciliate	s rotifers). Coel	lenterata: Actinaria: Not Applicable (e.g. fungi or algae studies) or Not Reported		
Health Outcome	Development/Growth					
Chemical:	Dibutyl nhthalate (DBP)					
HERO ID:	5495608					
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	The test substance was identified using electron-capture gas-liquid chromatography.		
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.		
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 initial placement of aquaria was determined.		
Domain 3: Exposure Ch	oractorization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-		
	Wieure 7.	Preparation	Wiedium	norted but there is some concern over the use of plastic travs		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups		
		Administration	ing.	Ziposalos vero administered consistenti actoss stady groupsi		
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.		
	Metric 10:	Concentration 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					
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.		
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.		
		Repleates per Group				
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 (harvesting) 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.		
Continued on next page						

Dibutyl Phthalate

continued from previous page					
Study Citation:	Tagatz, M. E	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic			
Duration:	communities Overall Dura	exposed to di-normal-butyl phthalate. Aquation: 11 - 21 days: Exposure Duration: 11 -	atic Toxicology (21 days	3(3):239-248.	
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study author	ors (i.e., chemica	l 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); Coel	enterata; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported	
Health Outcome:	Developmen	t/Growth			
Chemical:	Dibutyl phth	alate (DBP)			
	5495008				
Domain		Metric	Rating	Comments	
Domain 6: Confounding	y / Variable Co	atrol			
Domain of Contourianty	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	Medium	There was no information in the study to suggest differences among groups.	
Domain 7: Data Present	ation and Anal	voic			
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	Unexpected outcomes were satisfactorily explained.	
		* I	0	λ Ψ Ι.	
Additional Comments:	None				

Overall Quality Determination

Medium

Study Citation:	Tagatz, M. E communities	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration: Exposure Route, Media Path	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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) Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Echinodermata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth						
Taxa, Species, Age: Health Outcome:							
Chemical: HERO ID:	Dibutyl phth 5495608	Dibutyl phthalate (DBP) 5495608					
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	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
C	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 initial placement of aquaria was determined.			
Demain 2: Errorenne Ch							
Domain 3: Exposure Ch	aracterization	E	Madimu				
	Metric /:	Preparation	Medium	The experimental system and/or test media preparation methods were adequately re- ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	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 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.			
		Contin	nued on next pa	ge			

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HERO ID: 5495608 Table: 1 of 2

		contir	ued from previ	ous page			
Study Citation:	Tagatz, M. H	E., Deans, C. H., Moore, J. C., Plaia, G. R.	(1983). Alteratio	ons in composition of field-developed and laboratory-developed estuarine benthic			
	communities	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Echinodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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					
Domain 0. Comounding	Metric 19	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions			
	Mettre 17.	Design and Procedures	Ingn	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	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:	None						
Overall Qualit	ty Deterr	nination	Medium				

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Demotions	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Koute, Modio Dothy	Aquatic (marine); water; Not determined by study autors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	Lundebarte Other Innertebarte (a					
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Echinodermata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Chamical	Development/Growth					
HFRO ID:	Dibutyl phthalate (DBP)					
	5495008					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	Matria 1.	Test Substance Identity	Law			
	Metric 1: Matria 2:	Test Substance Identity	LOW	The test substance was identified using electron control and liquid character and the		
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-inquid chromatography.		
	Metric 3:	Test Substance Purity	LOW	Purity of the test substance was 100%.		
Domain 2: Test Design						
Domani 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		
			8	assessed outcomes.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-		
		Preparation	TT: 1	ported but there is some concern over the use of plastic trays.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered 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	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	U	response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organis	m Matria 12:	Test Organism Characteristics	Madium	Noturally coloniaino anomiamo years used and it is assumed there yes a secoliarity it.		
	Wieurie 15.	Test Organism Characteristics	Wiediulli	population structure.		
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-		
		Conditions		isms.		
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.		
		Replicates per Group				
Domain 5: Outcome Ag	aggmant					
Domain 5. Outcome As	Metric 16	A deguacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to avaluate if adapted		
	Metric 17:	Autquacy of Test Conditions	LOW	The outcome assessment methodology (harvesting) was not clearly reported		
	Metric 18	Consistency of Outcome	LOW	The outcome assessment methodology (harvesting) was not clearly reported.		
	withit 10.	Assessment	LOW	ited.		
		Conti	nued on next pa	π <u>ρ</u>		
Continued on next page						

		contin	ued from previ	ous page			
Study Citation:	Tagatz, M. E	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
	communities	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate;	Other Invertebrate (e.g., sea urchins, ciliate	s, rotifers); Echi	nodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
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.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
		D : (D	High	Data for exposure related findings were presented for each treatment and control group			
	Metric 22:	Reporting of Data	111211	Data for exposure-related infumes were presented for each treatment and control group.			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
	Metric 22: Metric 23:	Reporting of Data Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			

Study Citation:	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						
Duration: Exposure Route, Media. Path:	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)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 697762	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 697762					
Domain	Metric Rating Comments						
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The chemical was identified only by name. No other information was provided.			
	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 at least 99%.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative and solvent control group.			
	Metric 5:	Negative Control Response	High	The biological responses (percentage of cleavage, normal blastula, larval settlement and metamorphosis) of the negative control group were adequate.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
	, . <u>.</u> .						
Domain 3: Exposure Ch	Matria 7	Europimontal System/Test Madia	Madium	Made de ferrar antier effect medie en en de artheid in edemote des it hereiter start			
	Metric 7.	Propagation	Medium	taken to minimize loss of test substance was not reported			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups			
		Administration		Zaposalos 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 is not clear whether new test solutions were used for the incubation from swimming stage to the metamorphosis stage. Measured concentrations were similar to nominal concentrations.			
	Metric 10:	Exposure Duration and Frequency	High	The total duration of exposure was 96 hours. The experiment was done in two 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 spacing of exposure levels. 96 hr EC-50 values could not 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 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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Dibutyl Phthalate

		conti	nued from previ	ous page			
Study Citation:	Liu, Y., Guar abalone Hali	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.					
Duration:	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; Mollusks; Haliotis diversicolor supertexta; Embryo						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	697762						
Domain	Metric Rating Comments						
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers of test organisms and 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 As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health. DBP was found in the dilution medium (1,775 \pm 23 ng/l shown in Table 2).			
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes. The percentage of embryos undergoing cleavage and percentage of settled larvae were not sensitive endpoints. However, the percentage of normal blastula was a sensitive end point and 9hr EC50 values were calculated. The 96hr EC50 values, based on percentage of larvae that underwent metamorphosis, could not be calculated. Instead, 96hr NOEC values were reported.			
	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	8				
	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					
Domain 7. Data i lesen	Metric 21:	Statistical Methods	High	Statistical analysis was performed. ANOVA was used to test for differences among			

9hr EC50 values were reported based on the percentage of normal blastula and 96hr NOEC values were reported based on the percentage of larvae that underwent metamorphosis. For DBP, 96hr NOEC values were adjusted for background concentration of DBP in the dilution medium.

Reporting of Data

Explanation of Unexpected Outcomes

Overall Quality Determination

Additional Comments:

Metric 22:

Metric 23:

Medium

High

Medium

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

Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes. There was large within study variability for the settlement end point as

treatments and probit analysis was used to calculate 9hr EC50 values.

both normal and abnormal larvae settled.

Study Citation:	Yang, Z. H.,	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, Modia, Path:	Chinese Jour Overall Dura Aquatic (ma	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)					
Tava Snecies Age	Invertebrate	Invertebrate: Mollusks: Haliotis diversicolor supertexta: Larvae					
Health Outcome	Development/Growth						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
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	Low	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 response of the negative control groups was 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						
Domain of Exposure of	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were described in adequate detail but uncertainty over duration and concern for chemical loss caused downgrading.			
	Metric 8:	Consistency of Exposure	High	Details of exposure administration were reported and exposures were administered			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposure duration was to trochophore stage, which was somewhat arbitrary.			
	Metric 11:	Number of Exposure Groups/	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	High	Exposure concentrations were at or below the water solubility limit, solvent aided.			
Domain 4: Test Organis	m						
U	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of 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 As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to maintenance of health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.			
	Continued on next page						

HERO ID: 1322103 Table: 1 of 1

		contin	ued from previo	bus page			
Study Citation:	Yang, Z. H.,	Zhang, X. J., Cai, Z. H. (2009). Toxic effect	ts of several phth	alate esters on the embryos and larvae of abalone Haliotis diversicolor supertexta.			
	Chinese Jour	Chinese Journal of Oceanology and Limnology 27(2):395-399.					
Duration:	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	ors (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;	Larvae				
Health Outcome:	Developmen	nt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1322103	1322103					
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported and consistently applied 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	There was no information in the study to suggest differences among groups for out- comes unrelated to exposures.			
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:	None						
Overall Quali	Overall Quality Determination Medium						

Study Citation:	Zhou, J., Cai	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 Pollu Overall Dura Aquatic (mat	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; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo Mechanistic-Cell signaling/function Dibutyl phthalate (DBP) 1249532						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1: Metric 2: Metric 3:	Test Substance Identity Test Substance Source Test Substance Purity	Medium Low High	Chemical identified by name The test substance identity was not analytically verified by the performing laboratory Purity reported at $>=98\%$			
Domain 2 [.] Test Design							
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 Preparation	High	The experimental system and methods for preparation of 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	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/	High	The number of exposure groups and 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 aided by solvent at an appropriate concentration			
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			
	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						
Domain J. Outcome As	Metric 16	Adequacy of Test Conditions	High	Organism environmental conditions were conductive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest			
		Cont	inued on nex	xt page			

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HERO ID: 1249532 Table: 1 of 3

continued from previous page						
Study Citation:	Zhou, J., Cai	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	mental Pollution 159(5):1114-1122.				
Duration: Exposure Doute	A quatic (ma	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Media. Path:	Aquatic (marme); water, Not determined by study autiors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Taxa, Species, Age:	Invertebrate:	Mollusks: Haliotis diversicolor supertexta:	Embrvo			
Health Outcome:	Mechanistic-	Cell signaling/function				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1249532					
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	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 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:	Gene express	sion				
Overall Quality Determination			High			

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HERO ID: 1249532 Table: 2 of 3

Taxa, Species, Age: Invertebrate; Mollusks; Haliotis diversicolor supertexta; Embryo Health Outcome: Mechanistic-Oxidative stress (including redox biology) Chemical: Dibutyl phthalate (DBP) HERO ID: 1249532 Domain Metric Domain 1: Test Substance Metric 1: Metric 2: Test Substance Identity Metric 3: Test Substance Source Low The test substance identity was not analytically verified by the performing laboratory Metric 3: Test Substance Purity Metric 4: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation Low 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 Characterization Low Metric 7: Experimental System/Test Media Preparation High Preparation Metric 8: Consistency of Exposure Administration High Preparation Metric 9: Measurement of Test Substance Low Metric 9: Measurement of Test Substance<	Study Citation: Duration: Exposure Route, Media Path:	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ- 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)					
DomainMetricRatingCommentsDomain 1: Test SubstanceMetric 1:Test Substance IdentityMediumChemical identified by nameMetric 2:Test Substance SourceLowThe test substance identity was not analytically verified by the performing laboratoryMetric 3:Test Substance PurityHighPurity reported at >=98%Domain 2: Test DesignMetric 4:Negative ControlsHighStudy authors reported using appropriate concurrent negative control groupsMetric 5:Negative Control ResponseHighThe biological responses of the negative control groups were adequateMetric 6:Randomized AllocationLowResearchers did not report how organisms were allocated to study groupsDomain 3: Exposure CharacterizationMetric 7:Experimental System/Test Media PreparationHighMetric 8:Consistency of Exposure AdministrationHighThe experimental system and methods for preparation of test media were described in adequate detailMetric 9:Metric 07: Experimental System/Test Media PreparationHigh Consistency of ExposureHigh Consistency and methods for preparation of test media were described in adequate detailMetric 9:Consistency of Exposure AdministrationLowExposure concentrations were not measured consistently across study groupsMetric 10:ConcentrationLowExposure concentrations were not measured consistently across study groupsMetric 10:Exposure Duration and FrequencyHighThe duation of exposure and/or exposure frequency were reported and appropriate for </th <th>Taxa, Species, Age: Health Outcome: Chemical: HERO ID:</th> <th colspan="6">Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i>; Embryo Mechanistic-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 1249532</th>	Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo Mechanistic-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 1249532					
Domain 1: Test Substance Metric 1: Test Substance Identity Medium 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 at >=98% Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using appropriate concurrent negative control groups were adequate Metric 5: Negative Control Response High The biological responses of the negative control groups were adequate Domain 3: Exposure Characterization Low Researchers did not report how organisms were allocated to study groups 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 Details of exposure administration were reported and exposures were administered consistently across study groups Metric 9: Metasurement of Test Substance Low Exposure concentrations were not measured Metric 10: Exposure Duration and Frequency High The experimental system and nethods for preparation of test media appropriate for	Domain		Metric	Rating	Comments		
Metric 1: Test Substance Identity Medium 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 at >=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 Characterization Metric 7: Experimental System/Test Media Preparation High Metric 9: Consistency of Exposure High Details of exposure administration were reported and exposures were administered consistently across study groups Metric 9: Measurement of Test Substance Low Exposure concentration Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate for	Domain 1: Test Substan	ice					
Metric 2: Metric 3: Test Substance Source Test Substance Purity Low High The test substance identity was not analytically verified by the performing laboratory Purity reported at >=98% Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using appropriate concurrent negative control groups Metric 5: Negative Control Response 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 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/or exposure frequency were reported and appropriate for		Metric 1:	Test Substance Identity	Medium	Chemical identified by name		
Metric 3: Test Substance Purity High Purity reported at >=98% Domain 2: Test Design Metric 4: Negative Controls High Study authors reported using appropriate concurrent negative control groups were adequate 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 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 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 Concentration Concentration Low Exposure concentration of exposure and/or exposure frequency were reported and appropriate for		Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory		
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 Characterization High The experimental system and methods for preparation of test media were described in adequate detail 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 Details of exposure administration were reported and exposures were administered consistently across study groups Metric 9: 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		Metric 3:	Test Substance Purity	High	Purity reported at >=98%		
Metric 4: Metric 5: Metric 5: Metric 6:Negative Controls Negative Control Response Randomized AllocationHigh HighStudy authors reported using appropriate concurrent negative control groups Researchers did not report how organisms were allocated to study groupsDomain 3: Exposure CharacterizationKetric 7: PreparationExperimental System/Test Media PreparationHigh LowThe experimental system and methods for preparation of test media were described in adequate detailMetric 8: Metric 9:Consistency of Exposure Metric 10:High Exposure Duration and FrequencyHigh HighThe duration of exposure and/or exposure frequency were reported and appropriate for	Domain 2: Test Design						
Metric 5: Metric 6:Negative Control Response Randomized AllocationHigh LowThe biological responses of the negative control groups were adequate 		Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups		
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 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/or exposure frequency were reported and appropriate for		Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate		
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 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 and/or exposure frequency were reported and appropriate for		Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
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 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 Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate for	Domain 3. Exposure Ch	aracterization					
Preparation adequate detail Metric 8: Consistency of Exposure Administration High 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	Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in		
Metric 8: Consistency of Exposure Fign 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/or exposure frequency were reported and exposures were administered consistently across study groups		M. 4	Preparation	TT: -1-	adequate detail		
Metric 9: Measurement of Test Substance Low Exposure concentrations were not measured Concentration Concentration High The duration of exposure and/or exposure frequency were reported and appropriate for		Metric 8:	Administration	High	consistently across study groups		
Metric 10: Exposure Duration and Frequency High The duration of exposure and/or exposure frequency were reported and appropriate for		Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured		
the study type		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 spacing of exposure levels were adequate		Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and 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 aided by solvent at an appropriate concentration		Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit aided by solvent at an appropriate concentration		
Domain 4: Test Organism	Domain 4: Test Organis	m					
Metric 13: Test Organism Characteristics Low The source of the test animals was not reported	Domain +. Test 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 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms		
Conditions Medium The numbers of test organisms and replicates were reported and sufficient to character-ize toxicological effects Metric 15: Replicates per Group Medium The numbers of test organisms and replicates were reported and sufficient to character-ize toxicological effects		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	Domain 5: Outcome As	sessment					
Metric 16: Adequacy of Test Conditions High Organism environmental conditions were conductive to maintenance of organism health		Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conductive to maintenance of organism health		
Metric 17: Outcome Assessment Methodology High The outcome assessment methodology addressed the intended outcomes of interest		Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest		
Metric 18: Consistency of Outcome Assessment Consistency of Outcome High Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups		Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups		
Continued on next page			Cont	tinued on nex	xt page		

Environmental Hazard Evaluation

HERO ID: 1249532 Table: 2 of 3

continued from previous page							
Study Citation:	Zhou, J., Cai mental Pollu	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ- mental Pollution 159(5):1114-1122					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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;	Mollusks; Haliotis diversicolor supertexta	; Embryo				
Health Outcome:	Mechanistic	Oxidative stress (including redox biology)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1249532	1249532					
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			
	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	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:	MDA and Po	DD changes					
Overall Quality Determination High			High				

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HERO ID: 1249532 Table: 3 of 3

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: Exposure Route, Media, Path:	mental Pollu Overall Dura Aquatic (ma	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: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 1249532	Mollusks; <i>Haliotis diversicolor supertext</i> t/Growth alate (DBP)	a; Embryo				
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	Low	The test substance identity was not analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Purity was reported at $>=98\%$.			
Domain 2: Test Design							
2 Shain 2. Toot Dosigii	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 Preparation	High	The experimental system and methods for preparation of 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	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/	High	The number of exposure groups and 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 aided by solvent at an appropriate concentration.			
Domain 4. Test Organis							
Domain 4. 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 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 housing and environmental conditions were conductive to 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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HERO ID: 1249532 Table: 3 of 3

continued from previous page							
Study Citation:	Zhou, J., Cai mental Pollu	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone Haliotis diversicolor supertexta. Environ- mental Pollution 159(5):1114-1122.					
Duration:	Overall Dura	Overall Duration: 4 - 10 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;	Mollusks; Haliotis diversicolor supertexta;	Embryo				
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1249532						
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	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 Presentation and Analysis							
	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:	Comments: Metamorphosis rate, hatch rate, abnormality rate, surface structure changes						
Overall Quality Determination High							

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Study Citation:	Yang, Z. H.,	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:	Chinese Jour Overall Dura Aquatic (ma	Chinese Journal of Oceanology and Limnology 27(2):395-399. Overall Duration: Not-reported; Exposure Duration: Not-reported 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	; Embryo				
Health Outcome:	Developmen	t/Growth	•				
Chemical:	Dibutyl phth	alate (DBP)					
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							
C	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups was reported for abnormal devel- opment.			
	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 Preparation	Medium	The experimental system and methods for preparation of test media were described 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:	Concentration Exposure Duration and Frequency	Low	The exposure duration was to trochophore stage, somewhat arbitrary.			
	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	High	Exposure concentrations were at or below the water solubility limit with a solvent aid.			
Domain 4: Test Organis	m						
_ sinain rost organis.	Metric 13.	Test Organism Characteristics	Medium	There are minor reservations about the source of 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	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						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to maintenance of health.			
		Conti	nued on next pa	ge			
· · L.0							

HERO ID: 1322103 Table: 1 of 1

		contir	ued from previo	us page				
Study Citation:	Yang, Z. H., 2 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 Limpology 27(2):395-399.						
Duration:	Overall Dura	tion: Not-reported; Exposure Duration: No	t-reported					
Exposure Route,	Aquatic (mar	rine); Water; Not determined by study author	ors (i.e., chemical	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:	Development	t/Growth	•					
Chemical:	Dibutyl phtha	alate (DBP)						
HERO ID:	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 Assessment	Low	Somewhat subjective assessments were made, the criteria for abnormal development was not well defined.				
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 unrelated to test substance exposure.				
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	Abnormal was not well defined, and it was unclear which abnormalities were assessed.				
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.				
Additional Comments:	None							
Overall Qualit	y Detern	nination	Medium					

•	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic						
Duration: Exposure Route, Media, Path:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Mollusks; <i>Mollusca</i> ; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5495608						
Domain		Metric Rating Comments					
Domain 1: Test Substance	e		_				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
U	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 initial placement of aquaria was determined.			
Domain 2: Exposure Cha	reatorization						
Domain 5. Exposure Cha	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	Exposures were administered 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	High	The duration of exposure was suitable 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	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organism	n						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.			
Domain 5: Outcome Asse	essment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Contin	nued on next pa	ge			

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		contin	ued from previo	bus page			
Study Citation:	Tagatz, M. H	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration	Overall Dur	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
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 untake route)					
Media. Path:	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:	Invertebrate: Mollusks: Mollusca: multiple species: Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth	rippireuore (e.g.,	Tangi of algae statios) of Not Reported			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608	5495608					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 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.			
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	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Tagatz, M. E communities	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration: Exposure Route, Media. Path:	Overall Dura Aquatic (mar	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Mollusks; <i>Mollusca</i> ; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5495608						
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	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
8	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 initial placement of aquaria was determined.			
Domain 3: Exposure Characterization							
Domani et Enposare en	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately re- ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered 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	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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	response. Exposure concentrations were below the water solubility limit			
			6				
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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 (harvesting) 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.			
Continued on next page							

	continued from previous page						
Study Citation:	Tagatz, M. H	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
	communities	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate	Mollusks; Mollusca; multiple species; Not	Applicable (e.g.,	fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
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.			
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	Unexpected outcomes were satisfactorily explained.			
Additional Commentar	None						
Auditional Comments:	inone						
Overall Quality Determination Medium							

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 (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							
Chemical:	Dibutyl phth	alate (DBP)						
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 source was reported as provided by manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.				
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.				
Domain 2: Test Design	Matria 4	Negative Controls	High	A postive control was reported				
	Metric 4:	Negative Control Response	High	A negative control was reported.				
	Metric 5.	Pandomized Allocation	Low	An ellection method was not reported				
	Wieuric 0.	Kandonnized Anocation	LOW	All anocation method was not reported.				
Domain 3: Exposure Ch	aracterization							
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to pre- vent volatilization were not reported.				
	Metric 8:	Consistency of Exposure	High	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 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 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 water solubility.				
		· · · · · ·						
Domain 4: Test Organis	m		_					
	Metric 13:	Test Organism Characteristics	Low	A source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation 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 As	sessment	· · · ·						

Continued on next page ...
HERO ID: 1321996 Table: 1 of 1

		conti	nued from p	previous 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. E	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.						
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)							
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study auth	ors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Invertebrate;	Arthropods; Mysidopsis bahia; Not Appli	cable (e.g., fi	ungi or algae studies) or Not Reported				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
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	Outcome assessment was consistent across groups.				
		Assessment						
Domain 6: Confounding	g / Variable Cor	ntrol						
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.				
		Design and Procedures						
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.				
Domain 7: Data Present	ation and Anal	veic						
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	no unexpected outcomes were reported.				
Additional Comments:	None							
Overall Qualit	tv Detern	nination	High					

Study Citation:	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (ma	rine), Aquatic (brackish); Water; Not dete	rmined by stu	idy authors (i.e., chemical of interest in exposure water, but unable to determine exact		
Media, Path:	uptake route)				
Iaxa, Species, Age:	Invertebrate;	Arthropods; Mysidopsis bania; Juvenile				
Chemical	Dibutyl phth	alate (DBP)				
HERO ID:	1316220					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	се					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name, but no CASRN or structure were reported.		
	Metric 2:	Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA).		
	Metric 3:	Test Substance Purity	Low	Purity and/or substance were not included in the study.		
Domain 2: Test Design						
C C	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.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	The experimental design followed protocol guidelines.		
		Preparation	U			
	Metric 8:	Consistency of Exposure	High	Authors reported consistent adminstration.		
	Metric 9:	Administration Measurement of Test Substance	High	Phthalates were analytically verified and measured.		
	Metric 10.	Concentration Exposure Duration and Frequency	High	The test duration followed protocol		
	Metric 11:	Number of Exposure Groups/	High	The number of replicates used was adequate to the guidelines.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Concentrations used in the analysis were below the solubility limit.		
		~ .	U			
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	Medium	The source of organisms was reported, but details beyond that were not.		
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were housed for 1-3 days prior to treatment.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The replicates followed protocol.		
Domain 5: Outcome Ass	sessment					
	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.		
		Cont	inued on nex	t page		

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Dibutyl Phthalate

HERO ID: 1316220 Table: 1 of 1

continued from previous page						
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Bionomics,, Overall Dura Aquatic (ma uptake route Invertebrate; Mortality Dibutyl phth 1316220	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia). 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) Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Juvenile Mortality Dibutyl phthalate (DBP) 1316220				
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed and reported.		
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences were reported.		
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.		
Domain 7: Data Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistics performed included moving average angle analysis, probit analysis, and bino- mial probability.		
	Metric 22:	Reporting of Data	High	Data was reported adequately.		
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.		
Additional Comments:	None					
Overall Qualit	y Detern	nination	High			

Study Citation:	RB, Laughli grass 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 Pathy	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 upt						
Taxa, Species, Age:	Invertebrate	Invertebrate: Arthropods: PALAEMONETES PUGIO: Larvae					
Health Outcome:	ADME (biot	ADME (biotransformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333217						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The chemical was identified by accepted name [di-n-butyl phthalate (DBP)].			
	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							
Domain 21 Teor 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						
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and differences from nominal values varied 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 0 hours and after 24 hours. They were mea- sured via gas chromatography and 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 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 – test organisms were collected from the wild, with mini- mal 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 testing 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.			
		Contir	nued on next pa	ge			

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Environmental Hazard Evaluation

HERO ID: 1333217 Table: 1 of 3

		contin	ueu mom previ	ous page				
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:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Aquatic (mai	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; PALAEMONETES PUGIO; La	rvae					
Health Outcome:	ADME (biot	ransformation)						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1333217							
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 (75 larvae per concentration with three replicates).				
Domain 5: Outcome Ass	sessment	· · ·						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting environmental conditions was 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: 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.				
	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	Low	The study did not report any measures of variability, some variability was considered to be attributed to incomplete dissolution of test chemical				

Overall Quality Determination

Medium

Study Citation:	RB, Laughlin	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 purio (Holthuis). Water Air, and Soil Pollution 9(3):323–336.					
Duration:	Overall Dura	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:	Invertebrate;	Arthropods; PALAEMONETES PUGIO; La	rvae				
Chamical	Mortality	alata (DPD)					
HERO ID:	1333217						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		- C				
	Metric 1:	Test Substance Identity	High	Chemical was identified by accepted name [di-n-butyl phthalate (DBP)]			
	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 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						
	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations, differences from nominal values varied considerably			
	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	Low	Exposure concentrations were sampled at 0hr and after 24hr and measured via gas chro-			
		Concentration		matography but 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/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	response Exposure concentrations were at or below the water solubility limit, however droplets of			
	Metrie 12.	Testing at of Below Solubility Ellint	Low	chemical were noticed in test chambers			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations – test organisms were collected from the wild, with mini- mal 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 testing 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	Medium	The numbers of test organisms and realizates were reported and sufficient to character			
	wieute 15.	Replicates per Group	Mediuili	ize toxicological effects (75 larvae per concentration, three replicates)			
				• • • • • • • • • • • • • • • • • • • •			
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		conti	nued from previo	us page			
Study Citation:	RB, Laughli grass 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:	Overall Dura	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:	Invertebrate;	Arthropods; PALAEMONETES PUGIO; L	arvae				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333217						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting environmental conditions was 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 Assessment	High	Outcomes were assessed consistently across study groups			
Demain (+ Canfanadia	Warishla Car	1					
Domain of Contounding	Matria 10:	Confounding Variables in Test	Law				
	Methic 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			
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	Low	The study did not report any measures of variability, some variability was considered to be attributed to incomplete dissolution of test chemical			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route,	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. 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)				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 1333217	Arthropods; <i>PALAEMONETES PUGIO</i> ; Lat t/Growth alate (DBP)	rvae		
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	Chemical was identified by accepted name [di-n-butyl phthalate (DBP)]	
	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					
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				
-	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 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 and measured via gas chro- matography but 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 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. Toot Orregies					
Domain 4: Test Organisi	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations – test organisms were collected from the wild, with mini- mal 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 testing 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 numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects (75 larvae per concentration, three replicates)	
		Contin	ued on next pa	ge	

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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 pugio (Holthuip). Water, Air, and Soil Pollution 9(3):323–336.					
Duration:	Overall Dura	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:	1						
Taxa, Species, Age:	Invertebrate;	Arthropods; PALAEMONETES PUGIO; La	arvae				
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333217						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting environmental conditions was 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: 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			
	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	Low	Some variability was considered to be attributed to incomplete dissolution of test chemi cal			
Additional Comments:	None						

Study Citation: Duration:	Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study aut	hors (i.e., che	mical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 5557723	Arthropods; <i>Palaemonetes pugio</i> ; Not Apalate (DBP)	oplicable (e.g.	, fungi or algae studies) or Not Reported	
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	Medium	The authors state that "reagent grade" test chemicals were purchased from chemical supply companies.	
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 data from the biological responce of the negative control groups was not reported.	
	Metric 6:	Randomized Allocation	Low	Researchers did not report now organisms were allocated to study groups.	
Domain 3: Exposure Ch	aracterization				
Domain of Disposite of	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were described in adequate detail, but there is some concern over solubility. Glass beakers and jars were used in the bioassays.	
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9:	Administration Measurement of Test Substance	Low	Ranges of measured concentrations across a variety of experiments were reported, but	
	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 study does not report the number of exposure groups, what the exposure levels were, or the spacing of exposure levels. Only the highest concentration is reported in the	
	Metric 12:	Testing at or Below Solubility Limit	Low	results. The solvent concentration and biological response were not reported.	
Domain 4: Test Organis	m				
5	Metric 13:	Test Organism Characteristics	Medium	The test organisms were wild caught, with only a few in lab generations cultured.	
	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.	
		Replicates per Group			
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.	
		Cont	tinued on nex	xt page	

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HERO ID: 5557723 Table: 1 of 1

		conti	nued from p	previous page		
Study Citation: Duration: Exposure Route,	Clark, J. R., (Palaemonet Overall Dura Aquatic (mat	Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408. 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)				
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Palaemonetes pugio; Not Ap	plicable (e.g.	, fungi or algae studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5557723					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The intended outcome was mortality and was straightforwardly stated, but the details of the methodology such as the exposure concentrations, or the spacing of the exposure levels were not reported.		
	Metric 18:	Consistency of Outcome Assessment	Low	The details regarding the execution of the study protocol for outcome assessment were not reported.		
Domain 6: Confounding	g / Variable Coi	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	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:	The study do	bes not report the test's concentrations of e	xposure. It or	nly reports that no mortality (the endpoint of interest) occurred.		
Overall Quali	ty Detern	nination	Low			

Study Citation:	Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408.					
Duration: Exposure Route, Media Path	Overall Dura Aquatic (ma	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Aquatic (marine); 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:	Invertebrate; Mortality Dibutyl phth 5557723	Arthropods; <i>Palaemonetes pugio</i> ; Not Apalate (DBP)	oplicable (e.g.	, fungi or algae studies) or Not Reported		
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	Medium	The authors state that "reagent grade" test chemicals were purchased from chemical supply companies.		
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 experimental system and methods for preparation of test media were described in adequate detail, but there is some concern over solubility. Glass containers were used.		
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance	Low	Measured exposure concentrations 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/ Spacing of Exposure Levels	Low	The study does not report the number of exposure groups, what the exposure levels were, or the spacing of exposure levels. Only the highest concentration is reported in the results.		
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration was below solubility.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Medium	The organisms were wild caught, with only a few in lab generations cultured.		
	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, but duplicates were used.		
Domain 5: Outcome As	sessment		т			
	Metric 16:	Adequacy of lest Conditions	LOW	Environmental conditions were not sufficiently reported to evaluate if adequate.		
	Metric 17:	Outcome Assessment Methodology	LOW	i ne outcome assessment methodology was not clearly reported.		
		Cont	tinued on nex	at page		

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HERO ID: 5557723 Table: 1 of 1

continued from previous page						
Study Citation:	Clark, J. R., (Palaemonet	Clark, J. R., JR, Patrick, J. M., Jr, More, J. C., Lores, E. M. (1987). Waterborne and sediment-source toxicities of six organic chemicals to grass shrimp (Palaemonetes pugio) and amphioxus (Branchiostoma caribaeum). Archives of Environmental Contamination and Toxicology 16(4):401-408.				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Aquatic (ma	rine); Sediment; 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; Palaemonetes pugio; Not App	plicable (e.g.	, fungi or algae studies) or Not Reported		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5557723					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
Domain 6: Confounding	g / Variable Coi	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	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.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	The study di	d not report the exposure concentrations at	all. No mort	tality was observed at all.		
Overall Quali	ty Detern	nination	Low			

Study Citation: Duration: Exposure Route, Modia Bath:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 5495608	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Rhynchocoela</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5495608					
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	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
Domain 21 Teor Deorgi	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 initial placement of aquaria was determined.			
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 there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	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			
	Metric 12.	Spacing of Exposure Levels	High	response.			
	Wietrie 12.	Testing at of Delow Solubility Limit	Ingn	Exposure concentrations were below the water solubility innit.			
Domain 4: Test Organis	m						
C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ- isms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms 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.			
		Contin	nued on next pa	ge			

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HERO ID: 5495608 Table: 1 of 1

	continued from previous page						
Study Citation:	Tagatz, M. I	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate	; Other Invertebrate (e.g., sea urchins, ciliate	es, rotifers); Rhyn	chocoela; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth	-				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495608						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) 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 Co	ntrol					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.			
		Design and Procedures	C				
	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	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:	None						
Overall Queli	ty Dotorr	nination	Modium				
Villan Yuan		1111au1011	TATCATAILL				

Study Citation: Duration:	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6. Overall Duration: Not-reported; Exposure Duration: Not-reported						
Exposure Route,	Aquatic (ma	rine); Water; Not determined by study autho	rs (i.e., chemical of intere	est in exposure water, but unable to determine exact uptake route)			
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; 1 Developmen Dibutyl phth 790153	Vegetation; Non-vascular Plants; <i>Dunaliella parva</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 790153					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	— • • • •	-				
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low High	The test substance was identified by name only. The source of the DBP was reported to be Sigma Aldrich in St. Louis, MO. The DBP was analyzed by HPLC to be sure no monohydrolysis had occurred.			
	Metric 3:	Test Substance Purity	High	The DBP was reported to be an analytical reagent grade.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the DBP concentrations and media.			
	Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the exposure, so it is difficult to determine if the exposures were administered consistently. The number of organisms added to each exposure were just reported to be within a range. The exact time duration was not reported for each test. It appeared that organisms counts were not always performed on the same day or at the same time from Figure 1.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentrations were measured throughout the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	The exposure duration for each test was not reported. Exposure durations can be esti- mated from Figure 1, but not all counts appeared to be conducted at the same time.			
	Metric 11:	Number of Exposure Groups/	Medium	There were only two exposure concentrations. This is less than is typical, but it was enough for a response			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported that all test concentrations were below the water solubility limit.			
Domain 4: Test Organis	m						
_	Metric 13:	Test Organism Characteristics	High	Test organisms were reported to be either from the Marine Biology Laboratory of Cali- fornia State University Long Beach stock cultures or from Carolina Biological Supply.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the study.			
Continued on next page							

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HERO ID: 790153 Table: 1 of 1

		cor	ntinued from previous	page				
Study Citation:	Acey, R., He	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the						
Duration:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported						
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:	Vegetation; 1	Non-vascular Plants; <i>Dunaliella parva</i> ; Not A	pplicable (e.g., fungi or	algae studies) or Not Reported				
Health Outcome:	Developmen	nt/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	790153							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and	Low	Test organism concentrations were reported to be somewhere between 200 and 700				
		Replicates per Group		organisms/uL. This was quite a bit of variation. All tests were performed in triplicate.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Test organisms were grown in continuous light at 26C in F2 media modified with artificial seawater. More information on light intensity would be ideal.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth of the organisms in terms of aggregation and growth rate.				
	Metric 18:	Consistency of Outcome Assessment	Medium	Organisms were counted with a hemocytometer. It appeared from Figure 1 that not all concentrations were counted at the same time.				
Domain 6: Confounding	r / 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	Uninformative	Study authors did not conduct statistical analysis. Data is in graph form and exact num- bers are hard to determine.				
	Metric 22:	Reporting of Data	High	Exposure data and control response data are reported in Figure 1 and appear adequate for the outcome of interest.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.				
Additional Comments:	This portion received an	This portion of the evaluation is on the growth of green algae D. parva during DBP exposure. Development/growth was chosen as the outcome. The study received an unacceptable ranking due to lack of information regarding exposure duration and due to lack of statistical analysis.						

Overall Quality Determination

Uninformative

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 Dibutyl phthalate (DBP) 3230225				
Domain		Metric	Rating	Comments	
Domain 1: Test Substanc	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 provided.	
	Metric 3:	Test Substance Purity	High	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 controls (acetone concentration (0.5 mL L-1)) and the negative control	
	Metric 5:	Negative Control Response	High	No adverse effects 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 test substance before and during the exposure for these degradable substances. A solvent (acetone) was used to facilitate the prepara- tion of the stock solution.	
	Metric 8:	Consistency of Exposure Administration	Low	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 chemi- cals 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 is likely to be observed, the reported nominal concentrations are not likely to be representative of the final concentration and reporting in terms of nominal concentrations may underestimate 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	Medium	Exposure concentrations were reported to be 0, 1, 5, 10, 20, 30, 50, 100, 150, 200 mL/L. These appear to reflect the nominal concentration and no final test quantification was conducted, so these are likely not representative of the actual exposure concentration throughout the test. The number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.	

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Environmental Hazard Evaluation

HERO ID: 3230225 Table: 1 of 2

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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 Dibutyl phthalate (DBP) 3230225				
Domain		Metric	Rating	Comments	
	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.).	
Domain 4: Test Organi	sm				
6	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 organisms (starting algal density) was not reported.	
Domain 5: Outcome A	ssessment				
	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 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 significant effects were observed on growth following exposure to some of the test materials, but did not provide the measures of significance for each test concentration, so it was not clear what levels elicited these inhibitions on growth. There appeared to be a dose- response based on the graphs provided. This is likely to have a substantial impact on results.	
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments appears to be consistent across group. Cell numbers were deter- mined every 24 hours.	
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	ntation and Anal Metric 21:	lysis 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.	
		Cont	muea on neg	xt page	

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continued from previous page				
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 Dibutyl phthalate (DBP) 3230225			
Domain		Metric	Rating	Comments
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group (Figure 1). The author's discussion of the growth inhibition data for each phthalate was not clear, and their conclusions were made without incorporating any discussion of statistical significance.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.
Additional Comments:	Additional Comments: The discussion of growth inhibition following exposure to DEHP, DIBP, BBP and DBP was lacking.			
Overall Quali	ty Detern	nination	Low	

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 Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 3230225				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	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, and no additional information was pro- vided.	
	Metric 3:	Test Substance Purity	High	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 controls (acetone concentration (0.5 mL L-1)) and the negative control.	
	Metric 5:	Negative Control Response	High	The biological response of the control group was reported for mechanistic endpoints, and was acceptable.	
	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 test substance before and during the exposure for these degradable substances. A solvent (acetone) was used to facilitate the prepara- tion of the stock solution.	
	Metric 8:	Consistency of Exposure Administration	Low	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 chemi- cals 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 is likely to be observed, the reported nominal concentrations are not likely to be representative of the final concentration and reporting in terms of nominal concentrations may underestimate 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 test).	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure concentrations were reported to be 0,10, and 30 mL/L-1 and three replicates were used. These appear to reflect the nominal concentrations and no final test quantification was conducted, so these are likely not representative of the actual exposure concentrations throughout the test.	
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Dibutyl Phthalate

Environmental Hazard Evaluation

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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 Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP)				
Domain		Metric	Rating	Comments	
	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.).	
Domain 4: Test Organis	sm				
	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 (initial algal density) was not reported, and three repli- cates were used.	
Domain 5: Outcome As	ssessment				
	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 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 (MDA content, SOD, CAT and free radicals) was not clearly reported. It was unclear whether methods were sensitive for the outcome of interest. The authors reported that significant effects were observed on MDA and antioxidant levels following exposure to some of the test materials, but did not provide the measures of significance for each test concentration, so it was not clear what levels elicited these inhibitions on growth. There appeared to be a dose-response based on the graphs provided, but limited test concentrations limit the utility of these conclusions. This is likely 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 (e.g., tim- ing of assessment across groups) were confusing. It was reported that algal cell numbers were counted but results were not provided.	
Domain 6: Confoundin	σ / Variable Co	ntrol			
Zoman o. 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 (e.g., infection) that could influence the outcome assessment.	
Domain 7. Data Presen	tation and Anal	lycic			
Domain 7. Data 110301	Metric 21:	Statistical Methods	Low	One-way ANOVA was adopted to determine the significant differences between ex-	

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perimental and control groups. The calculations and measures of significance were not

provided so no conclusions about a dose response could be made.

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continued from previous page						
Study Citation:	Liu, N., Wer	n, F., Li, F., Zheng, X., Liang, Z., Zheng, H.	(2016). Inh	nibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508.		
Duration:	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 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:	Mechanistic	-Oxidative stress (including redox biology)-	Photosynth	lesis		
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	3230225					
Domain		Metric	Rating	Comments		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings (MDA content, SOD, CAT, free radical concentra- tion) were shown for each treatment and control group (Figures 3, 4, 5 and 6), but algal density data were not provided.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.		
Additional Comments: The discussion of growth inhibition following exposure to DBP and BBP was lacking.				d BBP was lacking.		
Overall Quality Determination		Low				

Study Citation:	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami-					
Duration	nation and T	nation and Toxicology 25(1):75-78.				
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:	Vegetation;	Non-vascular Plants; Skeletonema costatum	ı; diatoms; Not A	pplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	nt/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789981					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		Ŧ			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and grade were not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Medium	A 0.2mL portion of DBP was added to each salinity level of NH-15. Dilutions of this were used to make the DBP concentrations. It is unclear if the 0.2mL portion of the DBP was prepared in lab or if it was manufactured that way. Experiments were conducted in 16 x 125 mm Pvrex brand, disposable test tubes with a polypropylene cap.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 16 x 125mm test tubes at 24c in a growth chamber with continuous light. The concentration of diatoms was not reported, leaving uncertainties in diatom concentration for each replicate. It was only stated that 5 ml of of S. costatum culture in logarithmic growth phase was added to each dilution.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were frozen and later analyzed to determine DBP concentrations. The methods used were not reported, and the actual concentrations were not reported. Concentrations were only reported as % of saturation.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure was reported to be for 4d, which is typical of this type of study.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were seven exposure levels for each salinity and a control. The spacing was ad- equate to see a response. Study authors did not report actual exposure concentrations. Concentrations were reported as percentage of saturation. The stock solution was re- ported to be saturated with DBP but the exact concentration was not reported		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. The stock solution was reported to be saturated with DBP, and thus would not have been over the water solubility limit.		
Domain 4. Test Organis	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the diatoms was not reported.		
		Cont	inued on next pa	ge		

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Dibutyl Phthalate

HERO ID: 789981 Table: 1 of 4

		contin	ued from previ	ious page		
Study Citation:	Medlin, L. K nation and T	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78				
Duration:	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:	Vegetation; 1	Non-vascular Plants; Skeletonema costatum;	diatoms; Not A	pplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	nt/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	/89981					
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	It was reported that the diatom cultures were "preconditioned" to each salinity level prior to the study.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	The concentration of diatoms used in each test chamber was not reported. There were three replicates for each treatment level.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Cultures were kept at 24C with continuous light at the appropriate salinity for the study being conducted. Artificial seawater (NH-15) was used. The culture medium used for nutrition was not reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-developement/growth.		
	Metric 18:	Consistency of Outcome Assessment	High	Relative chlorophyll concentrations were measured at the start of the test and daily thereafter using a fluorometer. Cultures were fixed and initial and final cell counts were taken as well.		
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There could potentially have been differences in diatom concentrations between study groups as this was not reported in the paper.		
	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	Data were fitted to a quadratic model. A two-way analysis of variance and the student t-test was used to analyze the data.		
	Metric 22:	Reporting of Data	High	Data was presented for all exposure concentrations and salinities as well as for the con- trol in Table 3. Figure 1 presented data for exposure responses only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability.		
Additional Comments:	This study w 0/00 salinity	vas on the effect of DBP on diatom growth a	t different salin	ities. A new evaluation was created for each salinity. This evaluation is for the 14		
Overall Oualit	tv Detern	nination	Medium			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route	Medlin, L. K nation and T Overall Dura Aquatic (ma	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78. 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 untake route).				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; 1 Developmen Dibutyl phth 789981	Aquatic (marine); water; Not determined by study autnors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Non-vascular Plants; <i>Skeletonema costatum</i> ; diatoms; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 789981				
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 DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and grade were 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.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	A 0.2mL portion of DBP was added to each salinity level of NH-15. Dilutions of this were used to make the DBP concentrations. It is unclear if the 0.2mL portion of the DBP was prepared in lab or if it was manufactured that way. Experiments were conducted in 16 x 125 mm Pyrex brand, disposable test tubes with a polypropylene cap.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 16 x 125mm test tubes at 24c in a growth chamber with continuous light. The concentration of diatoms was not reported, leaving uncertainties in diatom concentration for each replicate.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were frozen and later analyzed to determine DBP concentrations. The methods used were not reported, and the actual concentrations were not reported. Concentrations were only reported as % of saturation.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure was reported to be for 4d, which is typical of this type of study.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were seven exposure levels for each salinity and a control. The spacing was ad- equate to see a response. Study authors did not report actual exposure concentrations. Concentrations were reported as percentage of saturation. The stock solution was re- ported to be saturated with DBP, but the exact concentration was not reported.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. The stock solution was reported to be saturated with DBP, and thus would not have been over the water solubility limit.		
Domain 4. Test Organia	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the diatoms was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	It was reported that the diatom cultures were "preconditioned" to each salinity level		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	prior to the study. The concentration of diatoms used in each test chamber was not reported. There were three replicates for each treatment level.		
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Environmental Hazard Evaluation

HERO ID: 789981 Table: 2 of 4

		contin	ued from previ	ous page		
Study Citation:	Medlin, L. K nation and T	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78.				
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:						
Taxa, Species, Age:	Vegetation; 1	Non-vascular Plants; Skeletonema costatum;	diatoms; Not A	pplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789981					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Cultures were kept at 24C with continuous light at the appropriate salinity for the study being conducted. Artificial seawater (NH-15) was used. The culture medium used for nutrition was not reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-developement/growth.		
	Metric 18:	Consistency of Outcome Assessment	High	Relative chlorophyll concentrations were measured at the start of the test and daily thereafter using a fluorometer. Cultures were fixed and initial and final cell counts were taken as well.		
Domain 6: Confounding	y / Variable Co	ntrol				
Domain of Confounding	Metric 19:	Confounding Variables in Test	Medium	There could potentially have been differences in diatom concentrations between study		
		Design and Procedures		groups as this was not reported in the paper.		
	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	Data were fitted to a quadratic model. A two-way analysis of variance and the student t-test was used to analyze the data.		
	Metric 22:	Reporting of Data	High	Data was presented for all exposure concentrations and salinities as well as for the con- trol in Table 3. Figure 1 presented data for exposure responses only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability.		
Additional Comments:	This study w 0/00 salinity	vas on the effect of DBP on diatom growth a	tt different salini	ties. A new evaluation was created for each salinity. This evaluation is for the 22		

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Medlin, L. k	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 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:	Vacatation	Non vocculor Dionte, Cheloton en a costatum	diatama Nat A	nnliaghla (a a' funai an alaga studiog) an Nat Danantad			
Taxa, Species, Age: Hoolth Outcomo:	Developmen	Non-vascular Plants; Skeletonema costatum	; diatoms; Not A	ppincable (e.g., lungi or algae studies) or Not Reported			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	789981						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		т				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 5:	Test Substance Purity	LOW	The purity and grade were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	Medium	A 0.2mL portion of DBP was added to each salinity level of NH-15. Dilutions of this			
	Wette 7.	Preparation	Weddun	were used to make the DBP concentrations. It is unclear if the 0.2mL portion of the DBP was prepared in lab or if it was manufactured that way. Experiments were conducted in 16 x 125 mm Pyrex brand, disposable test tubes with a polypropylene cap.			
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 16 x 125mm test tubes at 24c in a growth chamber with continuous light. The concentration of diatoms was not reported, leaving uncertainties in diatom concentration for each replicate.			
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were frozen and later analyzed to determine DBP concentrations. The methods used were not reported, and the actual concentrations were not reported. Concentrations were only reported as % of saturation.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure was reported to be for 4d, which is typical of this type of study.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were seven exposure levels for each salinity and a control. The spacing was ad- equate to see a response. Study authors did not report actual exposure concentrations. Concentrations were reported as percentage of saturation. The stock solution was re- ported to be saturated with DBP, but the exact concentration was not reported.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. The stock solution was reported to be saturated with DBP, and thus would not have been over the water solubility limit.			
Domain 4. Test Organis	m						
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the diatoms was not reported.			
	Metric 14:	Acclimatization and Pretreatment	High	It was reported that the diatom cultures were "preconditioned" to each salinity level			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The concentration of diatoms used in each test chamber was not reported. There were three replicates for each treatment level.			
		Conti	nued on next pa	ge			

Environmental Hazard Evaluation

HERO ID: 789981 Table: 3 of 4

		contin	ued from previ	ous page			
Study Citation:	Medlin, L. K nation and T	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78.					
Evnosuro Douto	Aquatic (ma	Aquetic (marine): Water: Not determined by study outbors (i.e., shemical of interact in exposure water, but upoble to determine exact uptake route)					
Media Path	Aquatic (IIIa	time), water, not determined by study aution	is (i.e., chennea	Tor interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation:	Non-vascular Plants: Skeletonema costatum:	diatoms: Not A	nnlicable (e.g. fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth		pprovore (e.g., runge of algae statute) of respected			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	789981						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Cultures were kept at 24C with continuous light at the appropriate salinity for the study being conducted. Artificial seawater (NH-15) was used. The culture medium used for nutrition was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-developement/growth.			
	Metric 18:	Consistency of Outcome Assessment	High	Relative chlorophyll concentrations were measured at the start of the test and daily thereafter using a fluorometer. Cultures were fixed and initial and final cell counts were taken as well.			
Domain 6: Confounding	y / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There could potentially have been differences in diatom concentrations between study groups as this was not reported in the paper.			
	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	Data were fitted to a quadratic model. A two-way analysis of variance and the student t-test was used to analyze the data.			
	Metric 22:	Reporting of Data	High	Data for was presented for all exposure concentrations and salinities as well as for the control in Table 3. Figure 1 presented data for exposure responses only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability.			
Additional Comments:	This study w 0/00 salinity	vas on the effect of DBP on diatom growth a	t different salini	ties. A new evaluation was created for each salinity. This evaluation is for the 36			

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route	Medlin, L. K nation and T Overall Dura Aquatic (ma	Medlin, L. K. (1980). Effects of di-n-butyl phthalate and salinity on the growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami- nation and Toxicology 25(1):75-78. 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 untake route).				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; 1 Developmen Dibutyl phth 789981	Aquatic (marine); water; Not determined by study autnors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Non-vascular Plants; <i>Skeletonema costatum</i> ; diatoms; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 789981				
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 DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity and grade were 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.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	A 0.2mL portion of DBP was added to each salinity level of NH-15. Dilutions of this were used to make the DBP concentrations. It is unclear if the 0.2mL portion of the DBP was prepared in lab or if it was manufactured that way. Experiments were conducted in 16 x 125 mm Pyrex brand, disposable test tubes with a polypropylene cap.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 16 x 125mm test tubes at 24c in a growth chamber with continuous light. The concentration of diatoms was not reported, leaving uncertainties in diatom concentration for each replicate.		
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were frozen and later analyzed to determine DBP concentrations. The methods used were not reported, and the actual concentrations were not reported. Concentrations were only reported as % of saturation.		
	Metric 10:	Exposure Duration and Frequency	High	The exposure was reported to be for 4d, which is typical of this type of study.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were seven exposure levels for each salinity and a control. The spacing was ad- equate to see a response. Study authors did not report actual exposure concentrations. Concentrations were reported as percentage of saturation. The stock solution was re- ported to be saturated with DBP, but the exact concentration was not reported.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. The stock solution was reported to be saturated with DBP, and thus would not have been over the water solubility limit.		
Domain 4. Test Organia	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the diatoms was not reported.		
	Metric 14:	Acclimatization and Pretreatment	High	It was reported that the diatom cultures were "preconditioned" to each salinity level		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	prior to the study. The concentration of diatoms used in each test chamber was not reported. There were three replicates for each treatment level.		
	Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 789981 Table: 4 of 4

		contin	ued from previ	ous page			
Study Citation:	Medlin, L. K nation and T	K. (1980). Effects of di-n-butyl phthalate and oxicology 25(1):75-78.	l salinity on the	growth of the diatom Skeletonema costatum. Bulletin of Environmental Contami-			
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	ors (i.e., chemica	l of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; 1	Non-vascular Plants; Skeletonema costatum;	diatoms; Not A	pplicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	789981						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Cultures were kept at 24C with continuous light at the appropriate salinity for the study being conducted. Artificial seawater (NH-15) was used. The culture medium used for nutrition was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-developement/growth.			
	Metric 18:	Consistency of Outcome Assessment	High	Relative chlorophyll concentrations were measured at the start of the test and daily thereafter using a fluorometer. Cultures were fixed and initial and final cell counts were taken as well.			
Domain 6: Confounding	y / Variable Co	ntrol					
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Medium	There could potentially have been differences in diatom concentrations between study			
		Design and Procedures	1.10010111	groups as this was not reported in the paper.			
	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	Data were fitted to a quadratic model. A two-way analysis of variance and the student t-test was used to analyze the data.			
	Metric 22:	Reporting of Data	High	Data for was presented for all exposure concentrations and salinities as well as for the control in Table 3. Figure 1 presented data for exposure responses only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability.			
Additional Comments:	This study w 0/00 salinity	vas on the effect of DBP on diatom growth a	tt different salini	ties. A new evaluation was created for each salinity. This evaluation is for the 27			

Overall Quality Determination

Medium

Study Citation:	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.						
Duration: Exposure Route, Media, Path:	Overall Dura Aquatic (ma	Overall Duration: Not-reported; Exposure Duration: Not-reported 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:	Vegetation; 1 Developmen Dibutyl phth 790153	Vegetation; Non-vascular Plants; <i>Synechococcus lividus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 790153					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Sigma Aldrich in St. Louis, MO. The DBP was analyzed by HPLC to be sure no monohydrolysis had occurred.			
	Metric 3:	Test Substance Purity	High	The DBP was reported to be an analytical reagent grade.			
Domain 2: Test Design							
c	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and in Table 1 and was adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the DBP concentrations and media.			
	Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the exposure, so it is difficult to determine if the exposures were administered consistently. The number of organisms added to each exposure were just reported to be within a range. The exact time duration was not reported for each test. It appeared that organisms counts were not always performed on the same day or at the same time from Figure 1.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentrations were measured throughout the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	The exposure duration for each test was not reported. Exposure durations can be esti- mated from Figure 1, but not all counts appeared to be conducted at the same time.			
	Metric 11:	Number of Exposure Groups/	High	There were five exposure concentrations. This was adequate to observe a response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported that all test concentrations were below the water solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Test organisms were reported to be either from the Marine Biology Laboratory of Cali- fornia State University Long Beach stock cultures or from Carolina Biological Supply.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the study.			
		C	Continued on next page				

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Environmental Hazard Evaluation

HERO ID: 790153 Table: 1 of 1

		00	ontinued from previous	s page			
Study Citation:	Acey, R., He environment	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.					
Duration:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported					
Exposure Route,	Aquatic (mar	rine); Water; Not determined by study author	rs (i.e., chemical of inter	rest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; N	Non-vascular Plants; Synechococcus lividus;	Not Applicable (e.g., fu	ngi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	790153						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	Test organism concentrations were reported to be somewhere between 200 and 700 organisms/uL. This was quite a bit of variation. All tests were performed in triplicate.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Test organisms were grown in continuous light at 26C in F2 media modified with artifi- cial seawater. More information on light intensity would be ideal.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth of the organisms in terms of aggregation and growth rate.			
	Metric 18:	Consistency of Outcome Assessment	Medium	Organisms were counted with a hemocytometer. It appeared from Figure 1 that not all concentrations were counted at the same time.			
Domain 6: Confounding	n / Variable Cor	atrol					
Domain 0. Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	Weute 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 Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	Low	Study authors reported not conducting statistical analysis, but independent statistical analysis may be conducted from data in Table 1.			
	Metric 22:	Reporting of Data	High	Exposure data and control response data are reported in Figure 1 and appear adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in Table 1, but not in Figure 1.			
Additional Comments:	This portion study receive	This portion of the evaluation is on the growth of blue-green algae S. lividus during DBP exposure. Development/growth was chosen as the outcome. The study received an unacceptable ranking due to lack of information regarding exposure duration.					

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route,	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6. Overall Duration: Not-reported; Exposure Duration: Not-reported 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: Health Outcome: Chemical: HERO ID:	Vegetation; 1 Developmen Dibutyl phth 790153	Vegetation; Non-vascular Plants; <i>Thalassioria pseudomona</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 790153				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low High	The test substance was identified by name only. The source of the DBP was reported to be Sigma Aldrich in St. Louis. MO. The DBP		
			1	was analyzed by HPLC to be sure no monohydrolysis had occurred.		
	Metric 3:	Test Substance Purity	High	The DBP was reported to be an analytical reagent grade.		
Domain 2: Test Design						
2 oniuni 21 Test 2 eoign	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the diatoms were allocated into study groups.		
Domain 3: Exposure Ch	varacterization					
Domain 9. Exposure er	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the DBP concentrations and media.		
	Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the exposure, so it is difficult to determine if the exposures were administered consistently. The number of organisms added to each exposure were just reported to be within a range. The exact time duration was not reported for each test. It appeared that organisms counts were not always performed on the same day or at the same time from Figure 1.		
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the exposure concentrations were measured throughout the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	The exposure duration for each test was not reported. Exposure durations can be esti- mated from Figure 1, but not all counts appeared to be conducted at the same time.		
	Metric 11:	Number of Exposure Groups/	High	There were four exposure concentrations. This was adequate to observe a response.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Study authors reported that all test concentrations were below the water solubility limit.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	High	Test organisms were reported to be either from the Marine Biology Laboratory of Cali- fornia State University Long Beach stock cultures or from Carolina Biological Supply.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the study.		
		Continued on next page				

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Environmental Hazard Evaluation

HERO ID: 790153 Table: 1 of 1

		coi	ntinued from previous	page			
Study Citation:	Acey, R., He environment	Acey, R., Healy, P., Unger, T. F., Ford, C. E., Hudson, R. A. (1987). Growth and aggregation behavior of representative phytoplankton as affected by the environmental contaminant di-n-butyl phthalate. Bulletin of Environmental Contamination and Toxicology 39(1):1-6.					
Duration:	Overall Dura	Dverall Duration: Not-reported; Exposure Duration: Not-reported					
Exposure Route,	Aquatic (ma	rine); 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:	Vegetation; 1	Non-vascular Plants; Thalassioria pseudomon	a; Not Applicable (e.g.,	fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	nt/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	790153						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	Test organism concentrations were reported to be somewhere between 200 and 700			
		Replicates per Group		organisms/uL. This was quite a bit of variation. All tests were performed in triplicate.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Test organisms were grown in continuous light at 26C in F2 media modified with artifi- cial seawater. More information on light intensity would be ideal.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth of the organisms in terms of aggregation and growth rate.			
	Metric 18:	Consistency of Outcome	Medium	Organisms were counted with a hemocytometer. It appeared from Figure 1 that not all concentrations were counted at the same time.			
		A solution					
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	lysis					
	Metric 21:	Statistical Methods	Uninformative	Study authors did not conduct statistical analysis. Data is in graph form and exact num- bers are hard to determine.			
	Metric 22:	Reporting of Data	High	Exposure data and control response data are reported in Figure 1 and appear adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	This portion study receive	This portion of the evaluation is on the growth of the diatom T. pseudomona during DBP exposure. Development/growth was chosen as the outcome. The study received an unacceptable ranking due to lack of information regarding exposure duration and due to lack of statistical analysis.					

Overall Quality Determination

Dibutyl Phthalate

Uninformative

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,				
Duration: Exposure Route, Media. Path:	and sheepshe Overall Dura Aquatic (bra	Dverall 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)				
Taxa, Species, Age:	Vertebrate; F	ertebrate; Fish; Cyprinodon variegatus; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	ADME (biot	ransformation)				
HERO ID:	789995	alate (DBP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The chemical was identified by name, but no CASRN or structure were given.		
	Metric 2:	Test Substance Source	High	The test substance source's include 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	No control was reported.		
	Metric 5:	Negative Control Response	N/A	No control was reported.		
	Metric 6:	Randomized Allocation	Low	The allocation method into groups was not reported.		
Domain 3: Exposure Ch	aracterization					
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Medium	The experimental design for test preparation was described.		
		Preparation				
	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	A 24-hour exposure period was used		
	Metric 11:	Number of Exposure Groups/	Low	Standard deviations were reported as two.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Two concentrations were below the solubility limit.		
			8			
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	Characteristics were not described in the study.		
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were acclimatized for four days prior to phthalate exposure.		
	Metric 15:	Conditions Number of Organisms and	Low	There was a low number of exposure groups.		
		Replicates per Group				
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.		
	Metric 17:	Outcome Assessment Methodology	High	Methodology was addressed.		
		(Continued on next page .			
PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 789995 Table: 1 of 1

		сог	ntinued from previous	s page			
Study Citation:	Wofford, H.	W., Wilsey, C. D., Neff, G. S., Giam, C. S., No	eff, J. M. (1981). Bioad	ccumulation and metabolism of phthalate esters by oysters, brown shrimp,			
	and sheepshe	ind sheepshead minnows. Ecotoxicology and Environmental Safety 5(2):202-210.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Aquatic (bra	ckish); Water; Not determined by study autho	rs (i.e., chemical of int	erest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vertebrate; F	Fish; Cyprinodon variegatus; Not Applicable (e.g., fungi or algae stu	dies) or Not Reported			
Health Outcome:	ADME (biot	transformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	789995						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	Medium	There was consistency in outcome assessment.			
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	-				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.			
Domain 7: Data Present	ation and Anal	vsis					
	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 Qualit	ty Detern	nination	Uninformativ	e			

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 Dibutyl phthalate (DBP) 1316224				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High High	The test material was identified and a CASRN was given. The source was listed as EG&G Bionomics: Aquatic Toxicology Laboratory in Ware-	
	Metric 3:	Test Substance Purity	Low	ham, Massachusetts. No other information about the source was given. 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	High	The experimental system and methods for preparation of test media were described in adequate detail and accounted for the properties of the test material. For all low- solubility phthalates, enhanced mixing procedure was used, while for butyl benzyl ph- thalate exposure microbial degradation was accounted for with a cleaning procedure 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 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:	Exposure Duration and Frequency	High	The 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 at <10weeks old.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	A 96-hour acclimation period was reported.	
Continued on next page					

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Dibutyl Phthalate

... continued from previous page **Study Citation:** Bionomics, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow (Cyprinodon variegatus) (final report). **Duration:** 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 **Exposure Route**, Media, Path: uptake route) Taxa, Species, Age: Vertebrate; Fish; sheepshead minnow (Cyprinodon variegatus); Juvenile **Health Outcome:** Mortality Chemical: Dibutyl phthalate (DBP) **HERO ID:** 1316224 Domain Metric Rating Comments Number of Organisms and Metric 15: Low Only two replicates of 10 fish were used in each treatment. Replicates per Group Domain 5: Outcome Assessment Adequacy of Test Conditions Metric 16: High Organism housing, environmental conditions, food, water, and nutrients were conducive to 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 High Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Medium The study reported minor differences among the study groups with respect to environmental conditions or other non-treatment-related factors, but these are unlikely to Design and Procedures have a substantial impact on results. Authors reported that dissolved oxygen fell below guideline 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., 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 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 explained. Low DO did not have an affect on the outcome and high mortality in controls was not reported for this chemical. Additional Comments: Study report for the Sheepshead minnow test begins on pg 124 of the PDF. DEHP is referred to as phthalate 1H, Dibutyl Phthalate was referred to as 1C,

and Butyl Benzyl Phthalate was referred to as 1D.

Overall Quality Determination	High

Study Citation:	V Citation: Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus				
-	laevis frogs.	Toxicological Sciences 84(2):394-407.	-		
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 2	1 days		
Exposure Route,	Aquatic (fre	shwater), Aquatic (brackish); Water; Not	determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine	
Media, Path:	exact uptake	route)			
Taxa, Species, Age:	Vertebrate; A	Amphibian; Xenopus laevis; Larvae			
Health Outcome:	Mechanistic	-Endocrine toxicity			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	128004				
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.	
Domain 2: Test Design					
Domani 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative	
		C C	C	control containing 0.01% DMSO.	
	Metric 5:	Negative Control Response	High	The biological response of the negative controls was reported in the text under results.	
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewal were conducted every M-W-F.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.	
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.	
	Metric 11:	Number of Exposure Groups/	High	There were 5 exposure levels as well as a negative control and a solvent control. This was adequate to see a response	
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.	
Domain 4. Test Organis	m				
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.	
		Cont	inued on nex	t page	

Environmental Hazard Evaluation

HERO ID: 128004 Table: 1 of 4

		conti	nued from p	revious page			
Study Citation:	Lee, S. K., laevis frogs.	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (fre	shwater), Aquatic (brackish); Water; Not	determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine			
Media, Path:	exact uptake route)						
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Mechanistic-Endocrine toxicity						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	128004						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome ris	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plasma testosterone levels.			
	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.			
	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	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	Low	Data for the exposure related findings was reported in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.			
Additional Comments:	This portion mechanistic	of the evaluation was on the effect of D endocrine outcome was chosen.	BP on testos	sterone levels in Xenopus laevis. Plasma testosterone levels were measured, so th			

Overall Quality Determination

High

Study Citation:	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.				
Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Overall Dura Aquatic (fre exact uptake Vertebrate; A Developmen Dibutyl phth 128004	ation: > 21 days; Exposure Duration: > 21 shwater), Aquatic (brackish); Water; Not o route) Amphibian; <i>Xenopus laevis</i> ; Larvae t/Growth alate (DBP)	l days determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine	
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.	
Domain 2: Test Design	Matria 4.	Nagativa Controls	High		
	Metric 4:	Negative Controls	nign	control containing 0.01% DMSO.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 1.	
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.	
Damain 2. Easterne Ch					
Domain 3: Exposure Ch	Motrie 7	Experimental System/Test Media	Uich	Stool colutions ware monored in 100ml on her close bottles weekly by adding DDD	
	Meure 7.	Preparation	nigii	directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewals were conducted every M-W-F.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure levels as well as a negative control and a solvent control. This was adequate to see a response.	
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate, indicat- ing the concentration was appropriate.	
Domain 4. Toot Ore					
Domain 4: Test Organisi	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.	
Continued on next page					

Environmental Hazard Evaluation

HERO ID: 128004 Table: 2 of 4

		conti	nued from p	revious page			
Study Citation:	Lee, S. K., V laevis frogs.	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Aquatic (free	shwater), Aquatic (brackish); Water; Not o	determined b	y study authors (i.e., chemical of interest in exposure water, but unable to determine			
Media, Path:	exact uptake	exact uptake route)					
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Xenopus laevis; Larvae					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	128004						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5 [,] Outcome As	sessment						
Domain D. Outcome 715	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–body weights/development/growth.			
	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	High	There were no reported differences among the study groups in environmental conditions			
	Medie 19.	Design and Procedures	mgn	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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	High	Data for exposure related findings including control results can be found in Table 1.			
	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 development	of the evaluation was on the effect of D t/growth outcome was chosen.	BP on body	weight in Xenopus laevis. Body weights for each treatment were obtained, so the			
Overall Qualit	ty Determ	nination	High				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., V laevis frogs. Overall Dura Aquatic (free exact uptake Vertebrate; A Reproductive Dibutyl phth 128004	Veeramachaneni, R., D.N. (2005). Subchi Toxicological Sciences 84(2):394-407. titon: > 21 days; Exposure Duration: > 2 shwater), Aquatic (brackish); Water; Not route) Amphibian; <i>Xenopus laevis</i> ; Larvae e/Teratogenic alate (DBP)	ronic exposur 1 days determined b	re to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus by study authors (i.e., chemical of interest in exposure water, but unable to determine
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce			
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.
Domain 2: Tast Dasian				
Domani 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a negative control in the FETAX solution and a negative control containing 0.01% DMSO.
	Metric 5:	Negative Control Response	High	The biological response of the negative controls was reported in Figures 2,3, and 6, as well as in Tables 1 and 2.
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewals were conducted every M-W-F.
	Metric 8:	Administration	High	sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.
	Metric 11:	Number of Exposure Groups/	High	There were five exposure levels as well as a negative control and a solvent control. This was adequate to see a response
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.
Domain 4. Test Organis	m			
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.
		Cont	inued on nex	at page

Environmental Hazard Evaluation

HERO ID: 128004 Table: 3 of 4

		conti	nued from p	revious page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Lee, S. K., Y laevis frogs. Overall Dura Aquatic (fre exact uptake Vertebrate; A Reproductivy Dibutyl phth	Veeramachaneni, R., D.N. (2005). Subchr Toxicological Sciences 84(2):394-407. ation: > 21 days; Exposure Duration: > 21 shwater), Aquatic (brackish); Water; Not of route) Amphibian; <i>Xenopus laevis</i> ; Larvae e/Teratogenic halate (DBP)	onic exposur days determined b	re to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus by study authors (i.e., chemical of interest in exposure water, but unable to determine
Domain	128004	Metric	Pating	Comments
Domain	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-histopathological changes in reproductive organs.
	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 to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7. Data Present	ation and Anal	vsis		
2011111 / 2011 110501	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.
	Metric 22:	Reporting of Data	High	Data for exposure related findings including control results can be found in Tables 1 and 2 as well as in Figures 2,3 and 6. Other figures provided exposure related results only.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.
Additional Comments:	This portion effects on the	of the evaluation was on the effect of DBI e reproductive system in male frogs. Repro	P on spermat duction was	togenesis in Xenopus laevis. Histopathological analysis was performed to observe the therefore selected as the outcome of interest.

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater), Aquatic (brackish); 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 Dibutyl phthalate (DBP) 128004				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.	
	Metric 2:	Test Substance Source	Low	The source was reported to be Sigma Aldrich, and the lot number was reported, but it was not reported if the DBP was analytically verified.	
	Metric 3:	Test Substance Purity	High	The DBP was reported to be 99.8% pure.	
Domain 2: Test Design	Metric A:	Negative Controls	High	Study authors reported using a pagative control in the EETAY solution and a pagative	
	Metric 4.	Negative controls	mgn	control containing 0.01% DMSO.	
	Metric 5:	Negative Control Response	High	Mortality in the control groups were less than 10% throughout the exposure.	
	Metric 6:	Randomized Allocation	Medium	It was reported that tadpoles were randomly assigned to treatment groups.	
Domain 3: Exposure Ch	aracterization				
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	High	Stock solutions were prepared in 100mL amber glass bottles weekly by adding DBP directly to DMSO. Glass pipettes were used to add the proper quantity to the test tanks. It was then manually stirred. Renewal were conducted every M-W-F.	
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered con- sistently across study groups. The same solvent was used in all test concentrations and in the solvent control. All test concentrations were run until complete metamorphosis of the control organisms.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the exposure concentrations were measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be from stage 52 (3wks) to stage 66 (8wks). This is typical of amphibian studies.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels as well as a negative control and a solvent control. This was adequate to see a response.	
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used in this study, and the solvent control response was adequate indicat- ing the concentration was appropriate.	
Domain 1: Test Organia	m				
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	Breeding adults were reported to be from Nasco in Fort Atkinson, WI. Adults were bred in the laboratory performing the study, and the tadpoles were reared there. The tadpole stages were appropriate for the study.	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same as treatment conditions. Temperature and pho- toperiod were the same for each.	
		Cont	inued on nex	xt page	

Environmental Hazard Evaluation

HERO ID: 128004 Table: 4 of 4

		conti	nued from p	revious page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Lee, S. K., Y laevis frogs. Overall Dura Aquatic (fre exact uptake Vertebrate; A Mortality	Lee, S. K., Veeramachaneni, R., D.N. (2005). Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in Xenopus laevis frogs. Toxicological Sciences 84(2):394-407. Overall Duration: > 21 days; Exposure Duration: > 21 days Aquatic (freshwater), Aquatic (brackish); 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					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	128004	()					
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms for each test concentration were reported, but the number of replicates for each test concentration was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Tadpoles were housed at a loading rate of 2.5 tadpoles/L. They were kept at 20-24C with a 12:12 photoperiod. The organisms were fed daily after 96h in age throughout the study. A slurry was used for the tadpoles and pellets were used after metamorphosis.			
	Metric 17:	Outcome Assessment Methodology	Low	The mortality assessment was not described in the methods section.			
	Metric 18:	Consistency of Outcome Assessment	Low	How mortality was assessed was not reported.			
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.			
	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					
/ 2 au 1 100010	Metric 21:	Statistical Methods	High	StatView was used for statistical analysis. ANOVA was used as well as Tukey-Kramer post hoc test.			
	Metric 22:	Reporting of Data	Medium	Mortality was just briefly described in text in the results section. Cumulative percent mortalities per treatment group were given there but no data was shown in any table or figure.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study authors did not report any unexpected outcomes. No variability was shown for mortality data.			
Additional Comments:	This evaluat	ion is for the mortality assessment during t	he study.				
Overall Qualit	ty Detern	nination	High				

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Study Citation:	Sugawara, N	(. (1974). Toxic effect of a normal series of	f phthalate es	ters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-		
Duration: Exposure Route, Media. Path:	89. 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)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 1315792	Arthropods; Artemia salina; Embryo alate (DBP)				
Domain	1010772	Metric	Rating	Comments		
Domain 1: Test Substan	ce	metre	Runng	connicits		
	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 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	A 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					
Domain C. Exposure C.	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 was 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:	Exposure Duration and Frequency	Medium	Figure 1 is for 72-hour values. The text states 40 or 72 hours was the exposure duration.		
	Metric 11:	Number of Exposure Groups/	High	Three treatment levels were used with adequate spacing between them.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	The solvent concentration seemed high. "The concentration of Triton X-100 in the con- trol, 10 ppm, and 20-ppm solutions was adjusted to 10 ppm by adding this reagent."		
Domain 4: Test Organis	m					
8	Metric 13:	Test Organism Characteristics	Low	The source of the test eggs was not reported.		
	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	One or two mg of eggs were placed in test dishes. This seems like a wide range. No replicates were reported.		
Domain 5: Outcome As	sessment		_			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.		
		Cont	inued on nex	xt page		

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Environmental Hazard Evaluation

HERO ID: 1315792 Table: 1 of 1

		conti	nued from p	revious page		
Study Citation:	Sugawara, N	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:	oy. Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route.	Aquation (brackish) 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	Invertebrate	Arthropods: Artemia salina: Embryo				
Health Outcome	Mortality	Thunopous, Thenna saina, Emoryo				
Chemical.	Dibutyl phth	alate (DBP)				
HFRO ID.	1315792					
	1515792					
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	g / Variable Con Metric 19:	ntrol 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	vsis				
	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	Low	Data for exposure-related findings were presented for each treatment and control group in Figure 1. The legend for the graph is not included. The labeling of phthalate esters is incorrect. Cannot locate DBP on the graph.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.		
	Mortality of	shrimp aggs in the control was - 47% and l	DMD data aa	uld not he leasted in Figure 1		

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: Exposure Route, Media. Path:	Overall Dura Aquatic (bra	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)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; ADME (biot Dibutyl phth 789995	Mollusks; <i>Crassostrea virginica</i> ; Not Appli transformation) alate (DBP)	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, but no CASRN or structure were given.		
	Metric 2:	Test Substance Source	High	The test substance sources include Aldrich Chemical Company for the unlabeled phtha- late and California Bionuclear Corporation for the 14C-labeled phthalate.		
	Metric 3:	Test Substance Purity	Low	Purity of the chemical was not included in the study.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Uninformative	An experiment with a control to test the absorbance of empty oyster shells was con- ducted. However, no controls were used for the experiment that measured concentra- tions in 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. An allocation method for test groups was not reported.		
Domain 3: Exposure Ch	aracterization					
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for test preparation was described. Procedures to account 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		
	Metric 10 [.]	Exposure Duration and Frequency	Medium	A 24-hour exposure period was used		
	Metric 11	Number of Exposure Groups/	Low	Only two exposure groups were used		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Two concentrations were below the solubility limit.		
		· · · · ·	-			
Domain 4: Test Organis	m		-			
	Metric 13:	Test Organism Characteristics	Low	Characteristics were not described in the study.		
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were acclimatized for four days prior to phthalate exposure.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	A low number of exposure groups was used.		
Domain 5: Outcome Ass	sessment					

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 789995 Table: 1 of 1

		e	continued from previous pa	age		
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 Dura	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; Mollusks; Crassostrea virginica; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	transformation)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789995					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.		
	Metric 17:	Outcome Assessment Methodology	High	Methodology was addressed.		
	Metric 18:	Consistency of Outcome	Medium	Outcome assessment was consistent for all groups.		
		Assessment				
Domain 6: Confounding	g / Variable Cor	ntrol				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The concentrations during the 24-hr exposure period remained relatively constant with the exception of the oyster experiments. The concentrations decreased 30-70% 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 oyster shells.		
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.		
Domain 7: Data Present	ation and Anal Metric 21:	ysis 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	Medium	Study size was small but data was reported.		
		r a second composition of according				
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Uninformative			

	D: :					
Study Citation:	Dionomics, EO&O (1964). Acute toxicity of twerve pinimate esters to mysici simmin (Mysiciopsis bailing). Overall Duration: $0 = 4$ days (0.96b): Exposure Duration: $0 = 4$ days (0.96b)					
Fyposure Route	Aquatic (ma	rine) Aquatic (brackish): Water: Not deter	rmined by str	(0-901)		
Media Path	untake route)	innice by se	aly autions (i.e., chemical of interest in exposure water, but unable to determine exact		
Taxa, Species, Age:	Invertebrate:	Arthropods: <i>Mysidopsis bahia</i> : Juyenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1316220					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name, but no CASRN or structure were reported.		
	Metric 2:	Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA).		
	Metric 3:	Test Substance Purity	Low	Purity and/or substance were not included in the study.		
Domain 2: Test Design						
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.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental design followed protocol guidelines.		
	Metric 8:	Consistency of Exposure	High	Authors reported consistent adminstration.		
	Metric 9:	Administration Measurement of Test Substance	High	Phthalates were analytically verified and measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The test duration followed protocol.		
	Metric 11:	Number of Exposure Groups/	High	The number of replicates used was adequate to the guidelines.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Concentrations used in the analysis were below the solubility limit.		
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	Medium	The source of organisms was reported, but details beyond that were not.		
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were housed for 1-3 days prior to treatment.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The replicates followed protocol.		
Domain 5: Outcome Ass	sessment					
	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.		
		Cont	inued on nex	ct page		

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continued from previous 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	rine), Aquatic (brackish); Water; Not determ	nined by stu	udy authors (i.e., chemical of interest in exposure water, but unable to determine exact			
Media, Path:	uptake route)					
Taxa, Species, Age:	Invertebrate;	Arthropods; Mysidopsis bahia; Juvenile					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1316220						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed and reported.			
		Assessment					
Domain 6: Confounding	Variable Co	atral					
Domain of Contounding	y Variable Col Matria 10:	Confounding Variables in Test	High	No differences were reported			
	Metric 19.	Design and Procedures	mgn	No unterences were reported.			
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.			
Domain 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistics performed included moving average angle analysis, probit analysis, and bino- mial probability.			
	Metric 22:	Reporting of Data	High	Data was reported adequately.			
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.			
Additional Comments:	None						
Overall Qualit	y Detern	nination	High				

Study Citation: Duration: Exposure Route,	Linden, E., water organi Overall Dura Aquatic (bra	Bengtsson, B. E., Svanberg, O., Sundstron sms, the bleak (Alburnus alburnus) and the ation: 0 - 4 days (0-96h); Exposure Duratio ackish); Water; Not determined by study aut	n, G. (1979). The harpacticoid Nite n: 0 - 4 days (0-9 thors (i.e., chemic	e acute toxicity of 78 chemicals and pesticide formulations against two brackish ocra spinipes. Chemosphere 8(11-12):843-851. 6h) cal of interest in exposure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Invertebrate	; Arthropods; <i>Nitocra spinipes</i> ; Adult		
Health Outcome:	Mortality			
Chemical:	Dibutyl phth	ialate (DBP)		
HERO ID:	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	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	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 stated that "No control analyses for the actual substance(s) were made of the test solutions."
	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	Medium	The duration of exposure and exposure frequency were reported and suitable, but slightly longer than typical for the study type (96h).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	At least six concentrations were tested, but the range of exposure groups was not re- ported.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. However, the reported LC50, 1.7 mg/L, is below the solubility reported in the Final Scope for DBP (11.2 mg/L at 25C).
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	Low	It is unclear if test organisms were acclimatized to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	Twenty organisms with no replicates per treatment were used.
Domain 5: Outcome As	sessment	· · ·		

Continued on next page ...

HERO ID: 51937 Table: 1 of 1

		conti	nued from previo	us page			
Study Citation:	Linden, E., water organi	Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish vater organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days (0-96	h)			
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	; Arthropods; Nitocra spinipes; Adult					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	51937						
Domain		Metric	Rating	Comments			
	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	Details of the outcome assessment protocol were reported and seemed consistent.			
		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	Medium	There was no information in the study to suggest differences among groups.			
		*					
Domain 7: Data Present	ation and Anal	lysis					
	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 Table 3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Deterr	nination	Medium				

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:	and sheepsho Overall Dura Aquatic (bra	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)					
Taxa, Species, Age:	Invertebrate;	nvertebrate; Arthropods; Penaecus aztecus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Dibutyl phth	alate (DBP)					
	189993						
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, but no CASRN or structure were given.			
	Metric 2.	Test Substance Source	Ingn	late and California Bionuclear Corporation for the 14C-labeled phthalate.			
	Metric 3:	Test Substance Purity	Low	Purity of the chemical was not included in the study.			
Domain 2: Test Design							
	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	Low	Organisms were collected from Galveston Bay. Allocation method into exposure groups 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 design for test preparation was described. Measures taken to account 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 sciptillation			
	Metric 10:	Exposure Duration and Frequency	Medium	A 24-hour exposure period was used.			
	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	Two concentrations were below the solubility limit.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	Characteristics were not described in the study.			
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were acclimatized for four days prior to phthalate exposure.			
	Metric 15:	Conditions Number of Organisms and	Low	A low number of exposure groups was used.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.			
		C	Continued on next page .	••			

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Environmental Hazard Evaluation

HERO ID: 789995 Table: 1 of 1

		cont	inued from previou	s 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 (bra	ckish); 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:	Invertebrate;	Arthropods; Penaecus aztecus; Not Applicable	e (e.g., fungi or algae	studies) or Not Reported		
Health Outcome:	ADME (biot	transformation)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789995					
Domain		Metric	Rating	Comments		
-	Metric 17:	Outcome Assessment Methodology	High	Methodology was addressed.		
	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent for all groups.		
		Assessment				
Domain 6: Confounding	g / Variable Co	ntrol				
	Metric 19:	Confounding Variables in Test	High	No confounding variables were indicated in the assessment reported.		
		Design and Procedures				
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.		
Domain 7: Data Present	ation and Anal	veie				
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	High	Outcomes unrelated to exposure were not reported.		
Additional Comments:	None					

Overall Quality Determination

Uninformative

Dibutyl Phthalate

Study Citation:	Cornell Univ	versity (1031) Penort upon the toxicity of t	lasticizars				
Duration.	Overall Dura	Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days					
Exposure Route.	Terrestrial: N	Terrestrial; N/A (e.g., injection); Injection					
Media. Path:							
Taxa. Species. Age:	Vertebrate: Mammalian: <i>Capra hircus</i> : Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1332948						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the injection was not reported. The exposure was via a subcutaneous injection.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Injections ap- peared to be administered daily for 11 days, but no other information was provided on the administration.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.			
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.			
Domain 4: Test Organis	m						
in the state of game	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15kg. The breed of the goat was not reported either.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the goats were acclimated in any way to test conditions.			
Continued on next page							

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Dibutyl Phthalate

HERO ID: 1332948 Table: 1 of 7

		co	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Mortality Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality. Observations for mortality were made for the duration of the test and then one day after the last exposure.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The goat was observed daily for mortality.		
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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	vsis				
Domain 7. Dua Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.					
Overall Qualit	ty Detern	nination	Uninformative	2		

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Respiratory Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Respiratory Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	Low	The preparation of the DBP for the injection was not reported. The exposure was via a subcutaneous injection.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Injections appeared to be administered daily for 11 days, but no other information was provided on the administration.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.		
Domain 4: Test Organis	m					
5	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15kg. The breed of the goat was not reported either.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.		
		С	ontinued on next page .			

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Dibutyl Phthalate

HERO ID: 1332948 Table: 2 of 7

		coi	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; N Respiratory Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Respiratory Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was reported autopsy was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment limited. Little details on the autopsy protocol were reported other than autopsy was performed 1 day after the final injection.		
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. Autopsy was performed. It was reported the goat exposed to DBP did not have any visible injury in the autopsy. Little other information was reported. The goat exposed to substance 3 (not PECO relevant) reported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, liver, and ovaries as well as a change in body temperature. So it is inferred these systems were checked in the goat exposed to DBP.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Hepatic/Live Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Hepatic/Liver Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	Low	The preparation of the DBP for the injection was not reported. The exposure was via a		
		Preparation	2011	subcutaneous injection.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Injections ap- peared to be administered daily for 11 days, but no other information was provided on the administration.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.		
Domain 4: Test Organis	m					
C	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15kg. The breed of the goat was not reported either.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.		
		С	ontinued on next page .			

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Dibutyl Phthalate

HERO ID: 1332948 Table: 3 of 7

		col	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media. Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Hepatic/Live Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Hepatic/Liver Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16: Metric 17:	Adequacy of Test Conditions Outcome Assessment Methodology	Low	No information on environmental conditions was provided. The outcome assessment methodology was not clearly reported. It was reported autopsy		
		Sateshie Hissessment Methodology	2011	was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment limited. Little details on the autopsy protocol were reported other than autopsy was performed 1 day after the final injection.		
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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	ysis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. Autopsy was performed. It was reported the goat exposed to DBP did not have any visible injury in the autopsy. Little other information was reported. The goat exposed to substance 3 (not PECO relevant) reported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, liver, and ovaries as well as a change in body temperature. So it is inferred these systems were checked in the goat exposed to DBP.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1332948 Table: 4 of 7

Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Reproductive Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the two goats were allocated into study groups.		
Damain 2. Emmanue Ch						
Domain 5: Exposure Ch	Matria 7	Even animental System /Test Madia	Low			
	Metric 7.	Dramonation	LOW	subcutaneous injection		
	Metric 8.	Consistency of Exposure	Low	Reporting omissions are likely to have a substantial impact on results. Injections an-		
	Metrie 6.	Administration	Low	peared to be administered daily for 11 days, but no other information was provided on the administration.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to be sufficient time to assess the outcomes of inter- est.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.		
Domain 4: Test Organis	m					
C	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15 kg. The breed of the goat was not reported either.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.		
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Dibutyl Phthalate

HERO ID: 1332948 Table: 4 of 7

		CO	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Reproductive Dibutyl phth 1332948	Mammalian; <i>Capra hircus</i> ; Not Applicable (e. e/Teratogenic alate (DBP)	g., fungi or algae studies	s) or Not Reported		
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was reported autopsy was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited. Little details on the autopsy protocol were reported other than autopsy was per- formed one day after the final injection.		
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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation of negative cone organism information liver, and ova	ion was on the effect of DBP administered vi control as well as because there was only one n and no control. Autopsy was performed. It was reported. The goat exposed to substance a aries as well as a change in body temperature.	a subcutaneous injection organism used in the st was reported the goat ex 3 (not PECO relevant) re . So it is inferred these s	n to a goat. This study received an unacceptable ranking due to the lack tudy. No statistical analysis could be conducted because there was only posed to DBP did not have any visible injury in the autopsy. Little other ported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, ystems were checked in the goat exposed to DBP.		

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; N	versity, (1931). Report upon the toxicity of p ation: 11 - 21 days; Exposure Duration: 11 - N/A (e.g., injection); Injection	lasticizers. 21 days	
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Gastrointest Dibutyl phth 1332948	Mammalian; <i>Capra hircus</i> ; Not Applicable (e inal nalate (DBP)	e.g., fungi or algae studies	s) or Not Reported
Domain		Metric	Rating	Comments
Domain 1: Test Substar	ice			
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.
Domain 2. Test Design				
Domain 2. Tost Design	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.
Domain 3: Exposure Cl	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the injection was not reported. The exposure was via a subcutaneous injection.
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Injections ap- peared to be administered daily for 11 days, but no other information was provided on the administration.
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.
Domain 4: Test Organis	sm			
2 chian ii Tost Organi	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15kg. The breed of the goat was not reported either.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.

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Dibutyl Phthalate

HERO ID: 1332948 Table: 5 of 7

		coi	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media. Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Gastrointesti Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Gastrointestinal Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was reported autopsy was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment limited. Little details on the autopsy protocol were reported other than autopsy was performed 1 day after the final injection.		
Domain 6: Confounding	g / Variable Coi	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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. Autopsy was performed. It was reported the goat exposed to DBP did not have any visible injury in the autopsy. Little other information was reported. The goat exposed to substance 3 (not PECO relevant) reported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, liver, and ovaries as well as a change in body temperature. So it is inferred these systems were checked in the goat exposed to DBP.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; N	versity, (1931). Report upon the toxicity of p ation: 11 - 21 days; Exposure Duration: 11 - J/A (e.g., injection); Injection	lasticizers. 21 days	
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Immune/Hen Dibutyl phth 1332948	Mammalian; <i>Capra hircus</i> ; Not Applicable (en natological alate (DBP)	e.g., fungi or algae studies	s) or Not Reported
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.
Domain 2. Test Design				
Domain 2. Tost Design	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.
Domain 3: Exposure Ch	varacterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the injection was not reported. The exposure was via a subcutaneous injection.
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Injections ap- peared to be administered daily for 11 days, but no other information was provided on the administration.
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15 kg. The breed of the goat was not reported either.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.

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Dibutyl Phthalate

HERO ID: 1332948 Table: 6 of 7

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Study Citation: Duration: Exposure Route, Media. Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Immune/Her Dibutyl phth 1332948	Mammalian; <i>Capra hircus</i> ; Not Applicable (e. matological alate (DBP)	g., fungi or algae studie:	s) or Not Reported		
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was reported autopsy was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment limited. Little details on the autopsy protocol were reported other than autopsy was performed 1 day after the final injection.		
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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. Autopsy was performed. It was reported the goat exposed to DBP did not have any visible injury in the autopsy. Little other information was reported. The goat exposed to substance 3 (not PECO relevant) reported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, liver, and ovaries as well as a change in body temperature. So it is inferred these systems were checked in the goat exposed to DBP.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Cardiovascu Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Cardiovascular Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
C C	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control. Only one goat was injected with DBP. The other goat was injected with substance 3.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the 2 goats were allocated into study groups.		
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	Low	The preparation of the DBP for the injection was not reported. The exposure was via a		
	Metric 8:	Preparation Consistency of Exposure Administration	Low	subcutaneous injection. Reporting omissions are likely to have a substantial impact on results. Injections ap- peared to be administered daily for 11 days, but no other information was provided on the administration.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be one injection a day for 11 days at doses rang- ing from 5cc to 50cc. This appeared to sufficient time to assess the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 11 day period, but there was only one goat to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit. Exposures were reported in ccs. The concentration of the DBP being dispensed in the injection was not reported.		
Domain 4: Test Organis	m					
-	Metric 13:	Test Organism Characteristics	Low	The source of the goats and the age of the goats were not reported. They were simply reported to be 15kg. The breed of the goat was not reported either.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the goats were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one goat exposed to DBP in this study. There was also no control, so no comparison could be statistically made.		
		С	ontinued on next page .			

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Dibutyl Phthalate

HERO ID: 1332948 Table: 7 of 7

		coi	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media. Path:	Cornell Univ Overall Dura Terrestrial; N	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Injection				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; M Cardiovascu Dibutyl phth 1332948	Vertebrate; Mammalian; <i>Capra hircus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Cardiovascular Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was reported autopsy was performed, but it is unclear what was looked at for this.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment limited. Little details on the autopsy protocol were reported other than autopsy was performed 1 day after the final injection.		
Domain 6: Confounding	g / Variable Coi	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. Environmental conditions were not reported, nor was it reported if the goat was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via subcutaneous injection to a goat. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. Autopsy was performed. It was reported the goat exposed to DBP did not have any visible injury in the autopsy. Little other information was reported. The goat exposed to substance 3 (not PECO relevant) reported effects in the gastrointestinal tract, lymph nodes, heart rate, lungs, liver, and ovaries as well as a change in body temperature. So it is inferred these systems were checked in the goat exposed to DBP.					

Overall Quality Determination

Uninformative

Dibutyl Phthalate

Study Citation:	Bello, U. M	I., Madekurozwa, M.,-C, Groenewald, H.	B., Aire, T. A., .	Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult			
-	male Japane	ale Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and					
	Physiology -	'hysiology - Part C: Toxicology and Pharmacology 166:24-33.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; I	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Vertebrate: Avian: Coturnix coturnix: japonica: Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Endocri	ne toxicity-Repro	oductive/Teratogenic			
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2346127						
	2510127						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Hıgh	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%.			
Demain 2. Test D							
Domain 2: Test Design	N . · · ·		TT' 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 reported and reasonable.			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.			
Domain 2: Exposure Ch	aractarization						
Domain 5: Exposure Ch	Matria 7	Europimantal System/Test Madia	Low	The study and ideal with the ideal details on the account to be the account is the account is the account of the			
	Metric 7:	Experimental System/ Test Media	LOW	the study provided only limited details on the measures taken to appropriately prepare			
		Preparation		plastic containers			
	Metric 8:	Consistency of Exposure	Medium	Only general methods of exposure administration were reported so assessment of cosis-			
	intente or	Administration		tency was difficult to determine.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
		Concentration		- r			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type (30 days).			
	Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and spacing of exposure levels were suitable to observe			
		Spacing of Exposure Levels		differences among groups.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via gavage			
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 for two weeks.			
	Mate 15	Conditions	Medium				
	Metric 15:	Number of Organisms and	Medium	The number of test organisms were reported and sufficient to characterize toxicological affacts (00 hirds divided into six groups inclusive of control, or 15 hirds nor group)			
		Replicates per Group		encers (20 onus urvided into six groups inclusive of control, of 15 onus per group).			
Domain 5: Outcome As	sessment						
Domain 5. Outcome Als	Metric 16	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate			
		racquacy of rest conditions		Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Conti	inued on next pa	age			

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Environmental Hazard Evaluation

HERO ID: 2346127 Table: 1 of 3

		conti	nued from previo	us page		
Study Citation: Duration: Exposure Route, Madia Bath	Bello, U. M male Japane Physiology Overall Dura Terrestrial; F	Bello, U. M., Madekurozwa, M.,-C, Groenewald, H. B., Aire, T. A., Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 166:24-33. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Avian; <i>Coturnix coturnix</i> ; japonica; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Endocrine toxicity-Reproductive/Teratogenic Dibutyl phthalate (DBP) 2346127					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest (steroid analysis via immunoassay, DNA/mRNA analysis via rtPCR).		
	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	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 (One-way ANOVA with Tukey's after testing for normality and homogeneity of variance).		
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in Figures 1-5.		
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Unexpected outcomes (non-monotonic response) were not satisfactorily explained.		
Additional Comments:	None					
Overall Quality Determination			Medium			

Study Citation: Duration: Exposure Route, Media, Path:	Bello, U. M male Japane Physiology - Overall Dura Terrestrial; F	I., Madekurozwa, M.,-C, Groenewald, H. se quails (Coturnix coturnix japonica) test - Part C: Toxicology and Pharmacology 166 ation: > 21 days; Exposure Duration: > 21 Food/Diet; Dietary	B., Aire, T. A., A is following pre- 5:24-33. days	Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Reproductiv Dibutyl phth 2346127	Avian; <i>Coturnix coturnix</i> ; japonica; Not Ap e/Teratogenic halate (DBP)	plicable (e.g., fun	gi or algae studies) or Not Reported
Domain		Metric	Rating	Comments
Domain 1: Test Substar	Netrie 1	Trad Calendary on Identity	II: -h	
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#.
	Metric 2: Metric 3:	Test Substance Purity	Low High	Chemical purity 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	There were no abnormalities seen in the control testis tissue.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups
Domain 3: Exposure Cl	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. It was not reported whether solutions were prepared in glass or plastic containers.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment of cosis- tency 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/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable, however no response was reported.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via gavage.
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	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 were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome As	ssessment			
	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 Assessment	High	outcomes were assessed consistently across study groups.
		Cont	inued on next pa	

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HERO ID: 2346127 Table: 2 of 3

	continued from previous page				
Study Citation: Duration:	Bello, U. M male Japane Physiology - Overall Dura	Bello, U. M., Madekurozwa, M.,-C, Groenewald, H. B., Aire, T. A., Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 166:24-33. Overall Duration: > 21 days; Exposure Duration: > 21 days			
Exposure Route, Modia Dath:	Terrestrial; I	food/Diet; Dietary			
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Avian; <i>Coturnix coturnix</i> ; japonica; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 2346127				
Domain		Metric	Rating	Comments	
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.	
	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	ysis			
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22:	Reporting of Data	High	Histological changes were reported in section 3.3 and shown in Figure 5.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	unexpected outcomes (low concentration effects) were not satisfactorily explained.	
Additional Comments:	This evaluat	ion is for the histological assessment of testi	s tissue.		

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path:	Bello, U. M male Japane Physiology Overall Dura Terrestrial; F	I., Madekurozwa, M.,-C, Groenewald, H. se quails (Coturnix coturnix japonica) test Part C: Toxicology and Pharmacology 166 ation: > 21 days; Exposure Duration: > 21 Food/Diet; Dietary	B., Aire, T. A., A is following pre- 5:24-33. days	Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Developmen Dibutyl phth 2346127	Vertebrate; Avian; <i>Coturnix coturnix</i> ; japonica; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 2346127				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	Netria 1.	Trat Calendary on Identity	II: -h			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#		
	Metric 2: Metric 3:	Test Substance Purity	Low High	Chemical purity 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	Medium	The biological response of the negative control group was only reported in text		
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups		
Domain 3: Exposure Cl	naracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. It was not reported whether solutions were prepared in glass or plastic containers.		
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment of cosis- tency 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/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable, however no response was reported		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via gavage		
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		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms were reported and sufficient to characterize toxicological effects		
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	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		
		Cont	inued on next pa	ge		

Environmental Hazard Evaluation

HERO ID: 2346127 Table: 3 of 3

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path:	Bello, U. M male Japane Physiology - Overall Dura Terrestrial; F	Bello, U. M., Madekurozwa, M.,-C, Groenewald, H. B., Aire, T. A., Arukwe, A. (2014). The effects on steroidogenesis and histopathology of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 166:24-33. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Food/Diet; Dietary			
Taxa, Species, Age: Health Outcome: Chemical:	Vertebrate; Avian; <i>Coturnix coturnix</i> ; japonica; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP)				
Domain	2346127	Metric	Rating	Comments	
Domain & Confounding	Variable Ca	ntrol	6		
Domain of Confounding	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	vsis			
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described	
	Metric 22:	Reporting of Data	Low	Data were only reported for GSI, other weight results were mentioned in text only	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	unexpected outcomes (low concentration effects) were not satisfactorily explained	

Dibutyl Phthalate

Study Citation: Duration: Exposure Route, Media Path	DuPont, (19 Overall Dura Terrestrial; F	DuPont, (1949). Toxicity of dibutyl phthalate. Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Avian; Gallus domesticus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1332945						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name. CAS # was provided on the cover sheet for this report by that is not part of the authors work.			
	Metric 2:	Test Substance Source	Low	No source was listed for this test product. Uncertainty is present with the actual veri- fication of the DBP as a blinded study was conducted: "The Plasticizer samples were received identified only by numbers. Our interpretation of these numbers is based on verbal information only, but was checked up fairly successfully by a determination of the specific gravity of the samples."			
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	The stock colony of animals was used as a control but no control survival was reported and they did not perform sham treatments for the effects of handling and dose adminis- tration.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported other than they were reported to "appear normal"			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	haracterization	E/T/T/Mdi-	T				
	Metric 7:	Preparation	Low	test concentrations			
	Metric 8:	Consistency of Exposure	Low	One time dose but with few details provided			
	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	N/A	exposure was via gavage			
Domain 4: Test Organis	Matria 12:	Test Organism Characteristics	Low	The source of the test enimels use not reported			
	Metric 14:	Acclimatization and Pretreatment	LOW	The source of the test animals was not reported. The study did not report whether pretreatment conditions were the same for control and			
	1,10010 1 4 .	Conditions	LOW	exposed groups as treated animals were removed from the stock culture			
			Continued on next page				
			. 0				

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Dibutyl Phthalate

HERO ID: 1332945 Table: 1 of 2

		сог	ntinued from previous	s page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	DuPont, (1949). Toxicity of dibutyl phthalate. Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus domesticus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality	alata (DBD)		
HERO ID:	1332945			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and	Low	Individuals without replicates were used
		Replicates per Group		
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
	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 limited
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	Low	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 was not conducted as this was not the intent of the study.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment but control group effects were not quantitated.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments:	None			

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	DuPont, (1949). Toxicity of dibutyl phthalate. Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; N/A (e.g., injection); Injection					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Dibutyl phth 1332945	Vertebrate; Avian; <i>Gallus domesticus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 1332945				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	The chemical was identified by name. The CAS# was provided on the cover sheet for this report, but that is not part of the author's work.		
	Metric 2:	Test Substance Source	Low	No source was listed for this test product. Uncertainty is present with the actual veri- fication of the DBP as a blinded study was conducted: "The Plasticizer samples were received identified only by numbers. Our interpretation of these numbers is based on verbal information only, but was checked up fairly successfully by a determination of the specific gravity of the samples."		
	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	The stock colony of animals was used as a control but no control survival was reported and they did not perform sham treatments for the effects of handling and dose adminis-		
	Metric 5:	Negative Control Response	Low	tration. The biological response of the negative control groups was not reported other than they were reported to "appear 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	The study provided only limited details on the measures taken to appropriately prepare test concentrations.		
	Metric 8:	Consistency of Exposure Administration	Low	A one-time dose was used, but with few details provided.		
	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 spacing of exposure levels were suitable for a dose response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via injection.		
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 Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups as treated animals were removed from the stock culture.		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Individuals without replicates were used.		
		С	ontinued on next page .			

Dibutyl Phthalate

HERO ID: 1332945 Table: 2 of 2

		con	tinued from previou	s page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	DuPont, (19 Overall Dura Terrestrial; N Vertebrate; A Mortality Dibutyl phth 1332945	49). Toxicity of dibutyl phthalate. ation: 11 - 21 days; Exposure Duration: 0 - 4 d N/A (e.g., injection); Injection Avian; <i>Gallus domesticus</i> ; Not Applicable (e.g. nalate (DBP)	ays (0-96h) , fungi or algae studie	es) or Not Reported
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	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 (e.g., timing of assessment across groups) were limited.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19: Metric 20:	Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure	Low Low	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.
Domain 7: Data Present	tation and Anal Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	N/A Medium	Statistical analysis was not conducted as this was not the intent of the study. Data for exposure-related findings were presented for each treatment, but control group effects were not quantitated. The study did not report any measures of variability.
Additional Comments:	None	Explanation of Onexpected Outcomes	Low	The study and not report any measures of variability.
Overall Ouali	tv Deterr	nination	Uninformativ	7 e

Dibutyl Phthalate

Study Citation:	Cornell University, (1931). Report upon the toxicity of plasticizers.						
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:	Vertebrate; A	Vertebrate; Avian; Gallus gallus; cockerels; Juvenile					
Health Outcome:	Ocular and S	Sensory					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1332948						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media	Low	The preparation of the DBP for the capsule that was administered was not reported.			
		Preparation	-				
	Metric 8:	Consistency of Exposure	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels			
		Administration		were fed capsules daily for 10 days with dose increasing by 1.5cc per kg every other			
	Matria 0.	Maggurament of Test Substance	Low	uay. Study outhous did not concert if the test desse were measured			
	Metric 9.	Concentration	LOW	Study authors did not report if the test doses were measured.			
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to 10 days with dosed capsules being fed every day with an increase in dose by 1.5cc per kg of body weight every other day.			
	Metric 11:	Number of Exposure Groups/	Low	There were several different doses administered over an 10 day period, but there was			
		Spacing of Exposure Levels		only one cockerel to which they were administered. It's possible the effect of the doses			
				administered could have been cumulative.			
	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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other informa-			
	N		Ŧ	tion was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15.	Conditions Number of Organisms and	Uninformative	There was only one hird exposed in each chamical, which was insufficient for statistical			
	mente 15.	Replicates per Group	Omnormative	analysis.			
		Replicates per Group		······ 2 ····			

Continued on next page ...

Dibutyl Phthalate

HERO ID: 1332948 Table: 1 of 5

		co	ntinued from previous	page			
Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Ferrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Vertebrate; A	ertebrate; Avian; Gallus gallus; cockerels; Juvenile cular and Sensory					
Chemical: HERO ID:	Dibutyl phth 1332948	alate (DBP)					
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after a day 10 exposure period, but the protocol was not described in any way.			
Domain 6: Confounding	y / 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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	vsis					
Domain / Dua Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.			
	Metric 22:	Reporting of Data	Low	Results were described in the text only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.			
Additional Comments:	This evaluati day for this p the study. No	ion was on the effect of DBP administered vi period. This study received an unacceptable ra o statistical analysis could be conducted becau	a capsule to a chicken for anking due to the lack of use there was only one o	or 10 days. The dose increased by 1.5cc per kg body weight every other negative control as well as because there was only one organism used in rganism and no control.			
Overall Qualit	ty Detern	nination	Uninformative	e			

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Study Citation: Duration: Exposure Route, Media Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Skin & Conr Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Skin & Connective Tissue Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2: Metric 3:	Test Substance Durity	Low	The source of the DBP was not reported.		
	Wietrie 5.	Test Substance Fullty	LOW	The purity/grade of the DBF was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 10 days with dose increasing by 1.5cc per kg every other day.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 10 days with dosed capsules being fed every day with an increase in dose by 1.5cc per kg of body weight every other day.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 10 day period, but there was only one cockerel to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.		
Domain 4: Test Organis	m		Ŧ			
	Metric 13:	Test Organism Characteristics	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Continued on next page					

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Dibutyl Phthalate

HERO ID: 1332948 Table: 2 of 5

		сог	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Cornell Univ Overall Dura Terrestrial; F Vertebrate; A Skin & Conr Dibutyl phth 1332948	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Skin & Connective Tissue Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after a day 10 exposure period, but the protocol was not described in any way.		
Domain 6. Confounding	y / Variable Cou	atrol				
Domain 0. Comountaing	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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	veis				
Domain 7. Data Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluati day for this p the study. No	ion was on the effect of DBP administered via period. This study received an unacceptable ra o statistical analysis could be conducted becau	a capsule to a chicken for nking due to the lack of ise there was only one o	or 10 days. The dose increased by 1.5cc per kg body weight every other regative control as well as because there was only one organism used in organism and no control.		

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Musculoskel Dibutyl phth 1332948	/ertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Ausculoskeletal Dibutyl phthalate (DBP) .332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT: 1			
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2: Matria 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 5:	Test Substance Purity	LOW	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
8	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 10 days with dose increasing by 1.5cc per kg every other day.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 10 days with dosed capsules being fed every day with an increase in dose by 1.5cc per kg of body weight every other day.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 10 day period, but there was only one cockerel to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
Continued on next page						

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Dibutyl Phthalate

HERO ID: 1332948 Table: 3 of 5

		col	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Cornell Univ Overall Dura Terrestrial; F Vertebrate; A Musculoskel Dibutyl phth	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Musculoskeletal Dibutyl phthalate (DBP)				
HERO ID:	1332948					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after a day 10 exposure period, but the protocol was not described in any way.		
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluati day for this p the study. No	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.				

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; I	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Aortality Dibutyl phthalate (DBP) 332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT 1			
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 5.	Test Substance Fullty	LOW	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
8	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 10 days with dose increasing by 1.5cc per kg every other day.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 10 days with dosed capsules being fed every day with an increase in dose by 1.5cc per kg of body weight every other day.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over an 10 day period, but there was only one cockerel to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
Continued on next page						

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Dibutyl Phthalate

HERO ID: 1332948 Table: 4 of 5

		col	ntinued from previous	page	
Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Mortality Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The bird was monitored for mortality for 10 days.	
	Metric 18:	Consistency of Outcome Assessment	High	The bird was monitored for mortality for 10 days.	
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	tation and Anal	ysis			
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.	
	Metric 22:	Reporting of Data	Low	Results were described in the text only.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.	
Additional Comments:	This evaluat day for this p the study. No	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.			

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; I	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Gastrointest Dibutyl phth 1332948	/ertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Jastrointestinal Dibutyl phthalate (DBP) J332948				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		TT 1			
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 5.	Test Substance Fullty	LOW	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
8	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 10 days with the dose increasing by 1.5cc per kg every other day.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported as 10 days with dosed capsules being fed every day with an increase in dose by 1.5cc per kg of body weight every other day.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were several different doses administered over a 10-day period, but there was only one cockerel to which they were administered. It's possible the effect of the doses administered could have been cumulative.		
	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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
Continued on next page						

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Dibutyl Phthalate

HERO ID: 1332948 Table: 5 of 5

		coi	ntinued from previous	page	
Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; F	versity, (1931). Report upon the toxicity of pla ation: 4 - 10 days; Exposure Duration: 4 - 10 d Food/Diet; Dietary	isticizers. Jays		
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Gastrointesti Dibutyl phth 1332948	Avian; <i>Gallus gallus</i> ; cockerels; Juvenile inal alate (DBP)			
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after a day 10 exposure period, but the protocol was not described in any way.	
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	veis			
Domain 7. Data Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.	
	Metric 22:	Reporting of Data	Low	Results were described in the text only.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.	
Additional Comments:	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. The study reported that the bird that received treatment one containing DBP, showed effects of a distended gall bladder and thickening of the small intestine.				
Overall Qualit	ty Detern	nination	Uninformative		

Dibutyl Phthalate

Study Citation:	Cornell University, (1931). Report upon the toxicity of plasticizers.							
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days					
Exposure Route,	Terrestrial; F	Food/Diet; Dietary						
Media, Path:	No stala seta A							
Iaxa, Species, Age: Hoolth Outcomo:	Vertebrate; A	Musculoskeletal						
Chemical	Dibutyl phthalate (DBP)							
HERO ID:	1332948							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	stance							
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.				
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.				
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Low					
	Metric 7:	Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.				
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight				
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.				
Domain 4: Test Organia	m							
Domanii 4. Test Organiis	Metric 13:	Test Organism Characteristics	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.				

Domain 5: Outcome Assessment

Continued on next page ...

Dibutyl Phthalate

HERO ID: 1332948 Table: 1 of 8

		co	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; F	ornell University, (1931). Report upon the toxicity of plasticizers. verall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days errestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Musculoskel Dibutyl phth 1332948	Avian; <i>Gallus gallus</i> ; cockerels; Juvenile letal lalate (DBP)				
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.		
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.					
Overall Quali	ty Detern	nination	Uninformative	9		

Study Citation: Duration: Exposure Route, Media Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Skin & Com Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Skin & Connective Tissue Dibutyl phthalate (DBP) 332948					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Ch	aracterization		-				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent 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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.			
				· · · · · · · · · · · · · · · · · · ·			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
Continued on next page							

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Dibutyl Phthalate

HERO ID: 1332948 Table: 2 of 8

		coi	ntinued from previous	page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Cornell Univ Overall Dura Terrestrial; F Vertebrate; A Skin & Conr Dibutyl phth 1332948	rersity, (1931). Report upon the toxicity of pla tion: 11 - 21 days; Exposure Duration: 11 - 2 ood/Diet; Dietary avian; <i>Gallus gallus</i> ; cockerels; Juvenile nective Tissue alate (DBP)	asticizers. 11 days		
Domain		Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.	
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	tation and Anal	vsis			
Domain 7. Data Proson	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.	
	Metric 22:	Reporting of Data	Low	Results were described in the text only.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.	
Additional Comments:	This evaluati day for this p the study. No	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.			

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Ocular and S Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Ocular and Sensory Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent 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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15:	Number of Organisms and	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical			
		Replicates per Group		anary 515.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
Continued on next page							

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Dibutyl Phthalate

HERO ID: 1332948 Table: 3 of 8

		col	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Cornell Univ Overall Dura Terrestrial; F Vertebrate; A Ocular and S Dibutyl phth 1332948	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Ocular and Sensory Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.		
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluati day for this <u>p</u> the study. No	ion was on the effect of DBP administered via period. This study received an unacceptable ra o statistical analysis could be conducted becau	a capsule to a chicken fo nking due to the lack of ise there was only one o	or 10 days. The dose increased by 1.5cc per kg body weight every other negative control as well as because there was only one organism used in rganism and no control.		

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Mortality Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT 1				
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Ch	naracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily until death, which was 11 days in the case of the DBP. The dosage was 1.8cc per kg administered daily.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported as 11 days (until death) with a dosage of 1.8cc DBP daily. This was sufficient for observations to be made.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.			
				<u>^</u>			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.			
		· · · ·					
Domain 5: Outcome As	sessment		Ŧ				
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
	Metric 1/:	Outcome Assessment Methodology	High	The bird was monitored for mortality for 11 days.			
Continued on next page							

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Dibutyl Phthalate

HERO ID: 1332948 Table: 4 of 8

		col	ntinued from previous	page	
Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Mortality Dibutyl phth 1332948	Avian; <i>Gallus gallus</i> ; cockerels; Juvenile alate (DBP)			
Domain	M-4	Metric	Rating	Comments	
	Metric 18:	Assessment	Hign	The bird was monitored for mortality for 11 days (until death occurred).	
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 or other non-treatment-related factors across study groups. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	vsis			
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.	
	Metric 22:	Reporting of Data	Low	Results were described in the text only in an unlabeled table.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.	
Additional Comments:	Comments: This evaluation was on the effect of DBP administered via capsule to a chicken for 11 days. The dose was reported to be 1.8cc per kg body weight until death. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control.				
Overall Qualit	ty Detern	nination	Uninformative		

Study Citation: Duration: Exposure Route, Media, Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Hepatic/Liv Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Hepatic/Liver Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	nce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Cl	naracterization		_				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.			
Domain 4. Tost Orregio							
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15:	Number of Organisms and Replicates per Group	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical analysis.			
				•			
Domain 5: Outcome As	sessment		Ţ				
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
		С	ontinued on next page .				

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Dibutyl Phthalate

HERO ID: 1332948 Table: 5 of 8

		col	ntinued from previous	page			
Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Hepatic/Live Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Hepatic/Liver Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.			
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	lycic					
Domain 7. Data Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.			
	Metric 22:	Reporting of Data	Low	Results were described in the text only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.			
Additional Comments:	ents: This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. The study reported that the affected bird showed effects on the gastrointestinal tract including the gizzard, stomach, small intestine, and gall bladder. Effects on other organ systems included the spleen, kidney, and liver.						
Overall Oualit	tv Deterr	nination	Uninformative	2			

Metric 11: Metric 12: m Metric 13: Metric 14: Metric 15: sessment Metric 16:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit Test Organism Characteristics Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group Adequacy of Test Conditions	N/A N/A Low Low Uninformative Low	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet. The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided. It was not reported if the birds were acclimated in any way to test conditions. There was only one bird exposed in each chemical, which was insufficient for statistical analysis. No information on environmental conditions was provided.		
Metric 11: Metric 12: Metric 13: Metric 14: Metric 15:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit Test Organism Characteristics Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	N/A N/A Low Low Uninformative	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet. The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other informa- tion was provided. It was not reported if the birds were acclimated in any way to test conditions. There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Metric 11: Metric 12: Metric 13: Metric 14: Metric 15:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit Test Organism Characteristics Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	N/A N/A Low Low Uninformative	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet. The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided. It was not reported if the birds were acclimated in any way to test conditions. There was only one bird exposed in each chemical, which was insufficient for statistical analysis.		
Metric 11: Metric 12: Metric 13: Metric 14:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit Test Organism Characteristics Acclimatization and Pretreatment Conditions	N/A N/A Low Low	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet. The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided. It was not reported if the birds were acclimated in any way to test conditions.		
Metric 11: Metric 12: m Metric 13:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit Test Organism Characteristics	N/A N/A Low	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet. The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
Metric 11: Metric 12:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A N/A	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet.		
Metric 11: Metric 12:	Number of Exposure Groups/ Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A N/A	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect. The exposure was via diet.		
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The exposure was conducted until death of the bird. There was only one exposure level. It did not appear that the goal was to observe a dose dependent effect.		
			The exposure was conducted until death of the bird.		
Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight.		
Metric 9:	Measurement of Test Substance	Low	weight. Study authors did not report if the test doses were measured.		
Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body		
Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
oractorization					
Metric 6:	Randomized Allocation	Low	was not reported. It was not reported how the chickens were allocated into study groups.		
Metric 4: Metric 5:	Negative Controls Negative Control Response	Uninformative	Study authors did not report the use of a negative control. Study authors did not report the use of a negative control, so a negative control response		
Matria 4.	Nagativa Controla	Lininformativa			
Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High Low	The DBP was identified by name and CASRN. The source of the DBP was not reported.		
ce					
	Metric	Rating	Comments		
Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Renal/Kidney Dibutyl phthalate (DBP) 1332948					
Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
	Cornell Univ Overall Dura Terrestrial; F Vertebrate; A Renal/Kidne Dibutyl phth 1332948 re Metric 1: Metric 2: Metric 2: Metric 3: Metric 3: Metric 5: Metric 5: Metric 6: aracterization Metric 7: Metric 8: Metric 9: Metric 10:	Cornell University, (1931). Report upon the toxicity of p Overall Duration: 11 - 21 days; Exposure Duration: 11 - Terrestrial; Food/Diet; Dietary Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Renal/Kidney Dibutyl phthalate (DBP) 1332948 <u>Metric 1</u> : Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Metric 5: 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	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary Vertebrate; Avian; Gallus gallus; cockerels; Juvenile Renal/Kidney Dibutyl phthalate (DBP) 1332948 <u>Metric 1: Test Substance Identity</u> Metric 2: Test Substance Source Low Metric 3: Test Substance Purity Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Metric 7: Experimental System/Test Media Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Low Concentration Metric 10: Exposure Duration and Frequency Metian 10: Exposure Duration and Frequency Metian 10: Exposure Duration and Frequency		

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Dibutyl Phthalate

HERO ID: 1332948 Table: 6 of 8

		coi	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media. Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Renal/Kidne Dibutyl phth 1332948	Avian; <i>Gallus gallus</i> ; cockerels; Juvenile y aalate (DBP)				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.		
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	veis				
Domain 7. Data i resent	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments:	This evaluat day for this j the study. No effects on th kidney, and	This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. The study reported that the affected bird showed effects on the gastrointestinal tract including the gizzard, stomach, small intestine, and gall bladder. Effects on other organ systems included the spleen, kidney, and liver.				
Overall Qualit	ty Deterr	nination	Uninformative			

Study Citation: Duration: Exposure Route,	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Gastrointestinal Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.		
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.		
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.		
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.		
Domain 3: Exposure Ch	naracterization		_			
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.		
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.		
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent 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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.		
	Metric 15:	Number of Organisms and	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical		
		Replicates per Group		unui yoio.		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.		
Continued on next page						

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Dibutyl Phthalate

HERO ID: 1332948 Table: 7 of 8

		coi	ntinued from previous	page			
Study Citation: Duration: Exposure Route, Media, Path:	Cornell Univ Overall Dura Terrestrial; F	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Gastrointesti Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Gastrointestinal Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.			
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Present	ation and Anal	veie					
Domain 7. Data Present	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.			
	Metric 22:	Reporting of Data	Low	Results were described in the text only.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.			
Additional Comments:	Its: This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. The study reported that the affected bird showed effects on the gastrointestinal tract including the gizzard, stomach, small intestine, and gall bladder. Effects on other organ systems included the spleen, kidney, and liver.						
Overall Oualit	tv Detern	nination	Uninformative	2			

Study Citation: Duration: Exposure Route, Media Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Immune/Her Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Immune/Hematological Dibutyl phthalate (DBP) 1332948					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by name and CASRN.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.			
	Metric 5:	Negative Control Response	Uninformative	Study authors did not report the use of a negative control, so a negative control response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chickens were allocated into study groups.			
Domain 3: Exposure Ch	aracterization		-				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the DBP for the capsule that was administered was not reported.			
	Metric 8:	Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. The cockerels were fed capsules daily for 11 days (until death) with at a dose of 1.8cc per kg of body weight.			
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the test doses were measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to 11 days with a dose of 1.8cc/kg body weight. The exposure was conducted until death of the bird.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level. It did not appear that the goal was to observe a dose dependent 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	Low	The source of the birds and the age were not reported. The chickens were reported to be cockerels that were still growing that weighed approximately 1.4kg. No other information was provided.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the birds were acclimated in any way to test conditions.			
	Metric 15:	Conditions Number of Organisms and	Uninformative	There was only one bird exposed in each chemical, which was insufficient for statistical			
		Replicates per Group		anaiysis.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	No information on environmental conditions was provided.			
Continued on next page							

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Dibutyl Phthalate

HERO ID: 1332948 Table: 8 of 8

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Study Citation: Duration: Exposure Route, Media. Path:	Cornell University, (1931). Report upon the toxicity of plasticizers. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; A Immune/Her Dibutyl phth 1332948	Vertebrate; Avian; <i>Gallus gallus</i> ; cockerels; Juvenile Immune/Hematological Dibutyl phthalate (DBP) 1332948				
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The chickens were examined after the study, but it was unclear what the protocols for this examination entailed.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cockerel was examined after death, which in the case of DBP, was an 11 day exposure period, but the protocol was not described in any way.		
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. Environmental conditions were not reported, nor was it reported if the cockerel was 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 Presenta	ation and Anal	vsis				
	Metric 21:	Statistical Methods	Uninformative	There was only one organism that was exposed to DBP and no control, so statistical comparison was not possible.		
	Metric 22:	Reporting of Data	Low	Results were described in the text only.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. There was only one organism exposed, so variability or unexpected outcomes were uncertain.		
Additional Comments: This evaluation was on the effect of DBP administered via capsule to a chicken for 10 days. The dose increased by 1.5cc per kg body weight every other day for this period. This study received an unacceptable ranking due to the lack of negative control as well as because there was only one organism used in the study. No statistical analysis could be conducted because there was only one organism and no control. The study reported that the affected bird showed effects on the gastrointestinal tract including the gizzard, stomach, small intestine, and gall bladder. Effects on other organ systems included the spleen, kidney, and liver.						
Overall Qualit	y Detern	nination	Uninformative			

Study Citation:	Abdul-Ghan (DEHP) and	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 Dura	ation: Not-reported; Exposure Duration: N	ot-reported					
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Injection						
Media, Path:	Vartabrata: A	vien: Callus callus domesticus: Cobb broi	ilor strain, En	nherro				
Hoolth Outcome:	Mortality	Mortality						
Chemical:	Dibutyl phthalate (DBP)							
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	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							
-	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/	N/A	Only one test concentration and a control group were used.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via egg injection.				
Domain 4: Test Organis	m							
station of guilds	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 Ass	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.				
		Conti	inued on nex	xt page				

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HERO ID: 1249807 Table: 1 of 3

		contin	ued from p	previous page		
Study Citation:	Abdul-Ghan (DEHP) and	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 Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported				
Exposure Route,	Terrestrial; N	J/A (e.g., injection); Injection				
Media, Path:						
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus gallus domesticus; Cobb broil	er strain; Ei	mbryo		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1249807					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	v / Variable Cou	ntrol				
Domain 0. Comounding	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 universes uniong the study groups in environmental conditions.		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no outcomes unrelated to exposures among test 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:	Mortality ad	ded for hatching.				
Overall Qualit	ty Detern	nination	High			

Study Citation:	Abdul-Ghani	i, S., Yanai, J., Abdul-Ghani, R., Pinkas, A di butul Phthalate (DBP) in a chick model	., Abdeen, Z.	(2012). The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate				
Duration:	Overall Dura	tion: Not-reported; Exposure Duration: N	ot-reported	biogy and relationgy 54(1).50-02.				
Exposure Route,	Terrestrial; N	VA (e.g., injection); Injection						
Media, Path:								
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Avian; Gallus gallus domesticus; Cobb broiler strain; Embryo						
Health Outcome:	Developmen	t/Growth						
Chemical: HERO ID.	1249807	alate (DBP)						
Domain	1219007	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	Chemical purity was reported as 99.7%.				
Domain 2: Test Design								
e 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	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
Domain of Enposure on	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/	N/A	Only one test concentration and a control group were used.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via egg injection.				
Domain 4. Test Organise								
_ shaan ii rost organisi	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 organism.				
	M . 15	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				
		Repleates 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.				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.				
		Assessment						
		Cont	inued on nex	t page				

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		contin	ued from p	previous page		
Study Citation:	Abdul-Ghan (DEHP) and	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 Dura	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; Eı	mbryo		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1249807					
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 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	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:	hatch, defect	S				
Overall Qualit	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: Exposure Route, Media Path	Overall Dura Terrestrial; N	erall Duration: Not-reported; Exposure Duration: Not-reported restrial; N/A (e.g., injection); Injection					
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus gallus domesticus; Cobb bro	iler strain; En	nbryo			
Health Outcome:	Mechanistic-	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1249807						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		TT: 1				
	Metric 1: Matria 2:	Test Substance Identity	High	The chemical substance was identified by name.			
	Metric 2:	Test Substance Source	LOW	The test substance identify was not analytically verified by the performing laboratory. The characteristic encounter $d = 0.0.7\%$			
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.7%.			
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	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		Ŧ				
	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			
	Wette 0.	Administration	mgn	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/	N/A	Only one test concentration and a control were used.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via egg injection.			
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	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					
		Cont	inued on nex	t page			

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		contin	ued from p	previous page	
Study Citation:	Abdul-Ghan	Abdul-Ghani, 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	d1-butyl Phthalate (DBP) in a chick model.	Neurotoxic	cology and Teratology 34(1):56-62.	
Exposure Route.	Terrestrial: N	J/A (e.g. injection): Injection	n-reported		
Media. Path:	Terrestriar, 1	(i.g., injection), injection			
Taxa, Species, Age:	Vertebrate; A	Avian; Gallus gallus domesticus; Cobb broil	ler strain; Ei	nbrvo	
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Genotox	(including	DNA repair)	
Chemical:	Dibutyl phth	alate (DBP)		•	
HERO ID:	1249807				
Domain		Metric	Rating	Comments	
Domain & Confounding	Variable Ca	ntanl			
Domain 6: Confounding	Matric 10:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions	
	Metric 19.	Design and Procedures	Ingn	There were no reported unreferces among the study groups in environmental conditions.	
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups reported.	
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:	DNA damag	e			
Overall Qualit	ty Detern	nination	High		

Study Citation:	Peakall, D.	B. (1974). Effects of di-n-butyl and di-2	2-ethylhexyl phthalate on the	e eggs of ring doves. Bulletin of Environmental Contamination and				
Duration:	Toxicology 1 Overall Dura	2(6):698-702. ation: Not-reported; Exposure Duration: N	ot-reported					
Exposure Route, Modia Pathy	Terrestrial; F	Food/Diet; Dietary						
Taxa, Species, Age:	Vertebrate; A	Avian; Streptopelia risoria; Adult						
Health Outcome:	Developmen	Development/Growth						
Chemical: HERO ID:	681729	alate (DBP)						
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	aracterization							
	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:	Exposure Duration and Frequency	Uninformative	The duration of exposure and exposure frequency were not reported.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Only one exposure group (10ppm DNB) was used to assess eggshell thickness, weight, rate of water loss, surface area and permeability. For DBP, eggshell thickness and weight were assessed for a group that were given 10 ppm DNBP diet followed by clean food (Table 1).				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.				
Domain 4: Trat Ora								
Domain 4: Test Organis	III Metric 13.	Test Organism Characteristics	Low	The course was not reported				
	Metric 13: Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study does not report whether pretreatment conditions were the same for control and exposed groups.				
			Continued on next page					

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Dibutyl Phthalate

		con	tinued from previou	s page			
Study Citation:	Peakall, D. Toxicology	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 12(6):698-702.					
Duration:	Overall Dura	ation: Not-reported: Exposure Duration: Not-re	eported				
Exposure Route.	Terrestrial: F	Food/Diet: Dietary	1				
Media. Path:	,	, and the second s					
Taxa, Species, Age:	Vertebrate: A	Avian: Streptopelia risoria: Adult					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	681729						
Domain		Metric	Rating	Comments			
	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).			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Pairs of ring doves were maintained as described in Peakall, 1970. 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 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 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:	No details gi experiment v	iven for the dietary toxicity testing methods. T vere not reported.	The duration of expos	ure and exposure frequency, and the number of pairs of doves used in the			

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Shin, N., Cu throughput s Overall Dura Terrestrial; C Invertebrate; Reproductive Dibutyl phth 5043459	enca, L., Karthikraj, R., Kannan, K., Colais creening in C. elegans. Pl o S Genetics 15(2 ation: 0 - 4 days (0-96h); Exposure Duration Cell Culture Media; Not determined by study Worms (e.g., Annelids, Nematodes); <i>Caence</i> e/Teratogenic talate (DBP)	ácovo, M. P. (20 2):e1007975. n: 0 - 4 days (0-9 y authors (i.e., ch prhabditis elegan	 Assessing effects of germline exposure to environmental toxicants by high- and the memory of the exposure water, but unable to determine exact uptake route) ans; Larvae
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			TT: 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 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	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	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 and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Four concentrations over an adequate range were 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			
c	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. An external publication was cited for standard culture methods.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Three replicate exposures were reported.

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5043459 Table: 1 of 4

		contin	ued from previ	ous page			
Study Citation:	Shin, N., Cu throughput s	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.					
Duration:	Overall Dura	verall 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;	Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Larvae					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5043459						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. The paper referred to "standard conditions" and cited an external source, and did not report conditions other than temperature (20C).			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest as described in the methods section, "Scoring embryonic lethality, larval lethality, and sterility"			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups as described in the methods			
		Assessment	_	section, "Scoring embryonic lethality, larval lethality, and sterility."			
Domain 6: Confounding	/ Variable Co	ntrol					
Bolliulii 0. Colliouliuliig	Metric 19.	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	metile 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.			
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:	Multiple stra	ains were tested, but it is unclear which strain adults were exposed to DBP. The reproductive	results were re	ported. This form is for reporting embryonic lethality, larval lethality, and progeny utcome was selected.			

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Study Citation: Duration:	Shin, N., Cu throughput s Overall Dura	enca, L., Karthikraj, R., Kannan, K., Colaiá creening in C. elegans. Pl o S Genetics 15(2 ation: 0 - 4 days (0-96h): Exposure Duration	ácovo, M. P. (20):e1007975. : 0 - 4 days (0-9	19). Assessing effects of germline exposure to environmental toxicants by high-			
Exposure Route,	Terrestrial; C	rrestrial; 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 Dibutyl phth 5043459	Worms (e.g., Annelids, Nematodes); <i>Caeno</i> Biomarkers (exposure and effect)-Cell signa alate (DBP)	orhabditis elegar aling/function-G	as; Larvae enotox (including DNA repair)			
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	Chemical 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 test substance was 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 reported and reasonable for assessed outcomes			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups. Multiple strains tested, unclear which strain results were reported for various tests			
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	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	Four concentrations over an adequate range			
	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 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			
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	High	The outcome assessment methodology reported the intended outcome of interest			
		Contin	nued on next pa	ge			

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Environmental Hazard Evaluation

HERO ID: 5043459 Table: 2 of 4

		contin	ued from previo	us 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-				
Duration	throughput screening in C. elegans. Pl o S Genetics 15(2):e1007975. Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route.	Terrestrial: (Overall Duration: 0 - 4 days (0-90n); Exposure Duration: 0 - 4 days (0-90n) Terrestrial: Cell Culture Media: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)				
Media, Path:	10110541141, (uutitois (itel, eli			
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Caeno	rhabditis elegan.	s; Larvae		
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell signa	aling/function-Ge	enotox (including DNA repair)		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5043459					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups		
Domain 6: Confounding	y / Variable Co	ntrol				
2 onium of Comountaing	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	Conditions There was no information in the study to suggest 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	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 tested, unclear which strain results were	reported			
Overall Quali	ty Detern	nination	Medium			

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HERO ID: 5043459 Table: 3 of 4

Study Citation:	Shin, N., Cu	uenca, L., Karthikraj, R., Kannan, K., Colaia	ácovo, M. P. (20	019). Assessing effects of germline exposure to environmental toxicants by high-			
Duration: Exposure Route, Modia Path:	throughput s Overall Dura Terrestrial; (Derestrial; 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> ; Larvae Mortality					
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	5043459						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical 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 test substance was 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 reported and reasonable for assessed outcomes			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups. Multiple strains tested, unclear which strain results were reported for various tests			
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	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/	High	Four concentrations over an adequate range			
	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 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. An external publication was cited for standard culture methods.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Three replicate exposures were reported.			
Domain 5: Outcome Ass	sessment						

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5043459 Table: 3 of 4

continued from previous page								
Study Citation:	Shin, N., Cu throughput s	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.						
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., ch	emical 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 elegan	s; Larvae				
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5043459							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. The paper referred to "standard conditions" and cited an external source, and did not report conditions other than temperature (20C).				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest as described in the methods section, "Scoring embryonic lethality, larval lethality, and sterility"				
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups as described in the methods section, "Scoring embryonic lethality, larval lethality, and sterility"				
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				
		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	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.				
Additional Comments:	Multiple stra	ins tested, unclear which strain results were	reported					
Overall Quality Determination			Medium					

buration: Overall Duration: 0 - 4 days (0-96h); Exposure at the second problem of the second problem					
Taxa, Species, Age: Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; Larvae Health Outcome: ADME (biotransformation) Chemical: Dibutyl phthalate (DBP) HERO ID: 5043459 Domain Metric Metric Test Substance Identity Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Domain 2: Test Design Metric 5: Metric 5: Negative Controls High Study authors reported using an appropriate concurrent negative control group Metric 6: Randomized Allocation Metric 6: Randomized Allocation					
Health Outcome: Chemical: Dibutyl phthalate (DBP)HERO ID:Dibutyl phthalate (DBP)5043459KetricDomainMetricRatingCommentsDomain 1: Test SubstanceTest Substance IdentityHigh Metric 2:Chemical identified by name and structureMetric 2:Test Substance SourceHigh HighChemical identified by name and structureMetric 3:Test Substance PurityLowPurity or grade of test substance was not reported.Domain 2: Test Design Metric 5:Metric 4:Negative Controls Regative Control ResponseHigh HighStudy authors reported using an appropriate concurrent negative control group Ametric 5:Metric 4:Negative Control ResponseHigh HighStudy authors reported using an appropriate concurrent negative control group Ametric 5:Metric 6:Randomized AllocationLowResearchers did not report how organisms were allocated to study groups. Multiple strains tested, unclear which strain results were reported for various tests					
Chemical: Dibutyl phthalate (DBP) HERO ID: 5043459 Domain Metric Rating Comments Domain 1: Test Substance Item in the substance Item in the substance Domain 1: Test Substance Identity High Chemical identified by name and structure Metric 1: Test Substance Identity High Chemical 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 test substance 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 Study authors reported using an appropriate concurrent 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 tested, unclear which strain results were reported for various tests					
HEROID: 5043459 Domain Metric Rating Comments Domain 1: Test Substance Metric 1: Test Substance Identity High Chemical 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 test substance 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 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 tested, unclear which strain results were reported for various tests					
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Metric 5. Test Substance Fullty Low Fullty of grade of test substance 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 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 tested, unclear which strain results were reported for various tests					
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Metric 6: Randomized Allocation Low Researchers did not report how organisms were allocated to study groups. Multiple strains tested, unclear which strain results were reported for various tests					
Domain 3: Exposure Characterization					
Metric /: Experimental System/Test Media 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					
Administration Admini					
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/ High Four concentrations over an adequate range					
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 Organism					
Metric 13: Test Organism Characteristics Low The original source was not reported					
Metric 14: Acclimatization and Pretreatment Conditions appeared to be the same for control and exposed organisms but few details were provided. An external publication was cited for standard culture methods.					
Metric 15: Number of Organisms and Replicates per Group Medium Three replicate exposures were reported.					
Domain 5: Outcome Assessment					
Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 5043459 Table: 4 of 4

continued from previous page								
Study Citation:	Shin, N., Cu throughput s	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.						
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., ch	emical 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 elegan	s; Larvae				
Health Outcome:	ADME (biot	ransformation)						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5043459							
Domain		Metric	Rating	Comments				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate. The paper referred to "standard conditions" and cited an external source, and did not report conditions other than temperature (20C).				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest as described in the methods section, "Scoring embryonic lethality, larval lethality, and sterility"				
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups as described in the methods				
		Assessment		section, "Scoring embryonic lethality, larval lethality, and sterility"				
Domain 6: Confounding	y / Variable Co	ntrol						
Domain of Comoundanty	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.				
	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:	Multiple stra	ins tested, unclear which strain results were	reported. This fo	orm is for reporting the ADME outcome in Fig 7 of the reference.				
Overall Quality Determination M			Medium					

May 2025

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					
Duration: Exposure Route, Media. Path:	AFD neuron Overall Dura Terrestrial; C	Diverall 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; Larvae					
Health Outcome:	Mechanistic-	-Oxidative stress (including redox biology))				
HERO ID:	2215375	alate (DBP)					
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 5:	Test Substance Purity	LOW	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 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 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 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 Effect level"			
	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	Low	The number of test organisms was not reported, but repeated tests were used as repli-			
		Replicates per Group		cates (n=5).			
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:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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HERO ID: 2215375 Table: 1 of 4

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	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.					
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	n: 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	legans; wild type; Larvae			
Health Outcome:	Mechanistic	Oxidative stress (including redox biology)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2215375						
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 that could influence the outcome assessment.			
Domain /: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Hıgh	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	High	There were no unexpected outcomes.			
Additional Comments:	Ethanol pret	reatment, reactive oxygen species					
Overall Quality Determination			High				

Study Citation: Duration: Exposure Route, Madia Data	Tseng, I. L., AFD neuron Overall Dura Terrestrial; C	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, Pain: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Dibutyl phth 2215375	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae Mechanistic-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 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							
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 Matria 12	Test Oreanism Chamatanistics	TT: _1.				
	Metric 13:	lest Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.			
	Metric 14:	Conditions	High	all 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: 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			
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups			
Continued on next page							

HERO ID: 2215375 Table: 2 of 4

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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:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2215375						
Domain		Metric Rating Comments					
Domain 6: Confounding / Variable Control							
	Metric 19:	Contounding 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	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 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:	Ascorbic aci	d pretreatment, reactive oxygen species					
Overall Quality Determination High							

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						
Duration: Exposure Route, Media, Path:	AFD neurons Overall Dura Terrestrial; C	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Perrestrial; 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); Caen	orhabditis e	elegans; DA 1267; Larvae			
Health Outcome:	Mechanistic-	Neurotoxicology-Ocular and Sensory					
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:	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 Organics	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			
	metric 11.	Conditions	mgn	an prodoution 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: 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	-				
Continued on next page							

HERO ID: 2215375 Table: 3 of 4

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	elegans. Pl	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 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:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	2215375					
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	High	there were no differences among groups that could influence the outcome assessment.		
Domain 7. Data Present	ation and Anal	vsis				
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		
				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:	AFD thermo	sensory neurons				
Overall Quali	ty Deterr	nination	High			

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae Behavioral Dibutyl phthalate (DBP) 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						
	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					
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:	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/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose		
		Spacing of Exposure Levels	U	response		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium		
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:	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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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

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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	elegans. Pl	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); Caena	orhabditis e	elegans; wild type; Larvae		
Health Outcome:	Behavioral					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	2215375	2215375				
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	High	there were no differences among groups that could influence the outcome assessment.		
	· 1 • 1					
Domain /: Data Present	ation and Anal	ysis	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 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: locomotor and thermotaxis					
Overall Qualit	ty Detern	nination	High			

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Study Citation:	Kang, S. W. Dermatopha	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides neuronysinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.						
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; N	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: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 485854	nvertebrate; Arthropods; <i>Dermatophagoides farinae</i> ; Adult Aortality Dibutyl phthalate (DBP) 185854						
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	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	No mortalities were reported in the negative control group.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.				
Domain 3: Exposure Ch	aracterization							
r	Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail. Petri dishes were used, but it was not mentioned whether they were made of glass or plastic.				
	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	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	Six concentrations were used.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The chemical was applied directly to cotton cloth.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	Organisms were from a lab culture, but origin of the colony was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Medium	Culture and test conditions were similar.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Three replicates of 30-40 adults were used.				
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. Temperature and relative humidity were reported.				
		Contin	ued on next pa	ge				

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HERO ID: 485854 Table: 1 of 2

continued from previous page						
Study Citation:	Kang, S. W., Dermatophag	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides netrophagoides (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.				
Duration:	Overall Dura	tion: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-90	5h)		
Exposure Route,	Terrestrial; N	/A (e.g., injection); Not determined by stud	y authors (i.e., cl	hemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides farinae; Ac	lult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	485854					
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 Con	trol				
-	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.		
Domain 7: Data Presenta	ation and Analy	zsis				
	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 tables.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					

Overall Quality Determination

Medium

Study Citation:	Kang, S. W. Dermatopha	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides nervous (Acari - Pyroglyphide). Journal of Agricultural and Food Chemistry 54(10):3547-3550					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Exposure Route,	Terrestrial; A						
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides farinae; A	dult				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	485854						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		т				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2: Matria 2:	Test Substance Source	Low	I he test substance identify was not analytically verified by the performing laboratory.			
	Metric 5.	Test Substance Fullty	LOW	Purity and/of grade of 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	No mortalities were 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 experimental system and methods for preparation of test media were described in adequate detail. Petri dishes were used, but it was not mentioned whether they were made of glass or plastic.			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported and exposures were administered consistently across study groups, but because it was a vapor exposure, consistency is difficult to judge.			
	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/	N/A	Only one concentration was used, although both open and closed systems were evalu-			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The chemical was applied directly to cotton cloth, and exposure was via vapors.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Organisms were from a lab culture, but origin of the colony was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Medium	Culture and test conditions were similar.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Three replicates of 30-40 adults were used.			
Domain 5: Outcome As	sessment						
2 omum 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health. Temperature and relative humidity were reported.			
	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: 485854 Table: 2 of 2

		continu	ued from previo	ous page			
Study Citation:	Kang, S. W. Dermatopha	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., ch	emical of intere	st in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides farinae; Ad	lult				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	485854						
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	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	Low	Statistical methods were adequately described but results were not compared to controls; they were only compared to other chemicals.			
	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:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air, 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) Invertebrate; Arthropods; <i>Dermatophagoides farinae</i> ; Adult Mortality Dibutyl phthalate (DBP) 1332803			
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	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	No control mortalities were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization Metric 7: Metric 8: Metric 9: Metric 10:	Experimental System/Test Media Preparation Consistency of Exposure Administration Measurement of Test Substance Concentration Exposure Duration and Frequency	Low Medium Low High	The study provided only limited details on the measures taken to appropriately prepare test concentrations. A one-time dose was used, but few details were provided. Exposure concentrations were not measured. The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	High	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fabric contact.
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 test used 25-35 mites with three replicates.
Domain 5: Outcome Ass	sessment Metric 16: Metric 17:	Adequacy of Test Conditions Outcome Assessment Methodology Conti	Medium High nued on next pa	Control and treatment mites were held in conditions similar to rearing conditions. The outcome assessment methodology addressed the intended outcome of interest. ge

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HERO ID: 1332803 Table: 1 of 2

	continued from previous page				
Study Citation: Duration: Exposure Route,	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air, N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake				
Media, Path:	route)		1.1.		
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides farinae; A	Adult		
Chemical: HERO ID:	Mortality Dibutyl phthalate (DBP) 1332803				
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	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	tation and Anal	ysis			
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not compared to control 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	High	There were no unexpected outcomes.	
Additional Comments:	closed conta	iner, method A			
Overall Quali	ty Detern	nination	Medium		

Study Citation:	Kim, H. K.,	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and				
	Dermatopha	Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861.				
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:	Inventabuata	Anthropoda, Dominator has sides faminas A	dul+			
Taxa, Species, Age:	Mortality	, Arunopous, Dermaiopnagoiaes jarinae, A	duit			
Chemical	Dibutyl phth	alate (DBP)				
HERO ID:	1332803					
 Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce	moure	Ruting	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	Matria 4.	Norther Controls	II: -h			
	Metric 4:	Negative Controls	Hign	Study authors reported using an appropriate concurrent negative control group.		
	Metric 5:	Negative Control Response	Medium	A biological response by a control group was reported in Table 1, but it's unclear if this was the response of the control mentioned in the methods section.		
	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		
		Preparation	2011	test concentrations.		
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used, but few details were provided.		
	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 exposure and/or exposure frequency were reported and appropriate for the study type.		
	Metric 11:	Number of Exposure Groups/	High	Eight concentrations over a suitable range were used.		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fabric contact.		
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		1		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The test used 25-30 mites with 3-5 replicates.		
		· · · ·				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Medium	Control and treatment mites were held in conditions similar to rearing conditions.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.		
	Metric 18:	Assessment	High	Outcomes were assessed consistently across study groups.		
		Conti	nued on next pa			

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HERO ID: 1332803 Table: 2 of 2

		contin	ued from previ	ous page	
Study Citation:	Kim, H. K.,	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and			
	Dermatopha	goides pteronyssinus (Acari: Pyroglyphidae). Journal of Ag	ricultural and Food Chemistry 52(26):7857-7861.	
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days (0-9	6h)	
Exposure Route,	Terrestrial; N	N/A (e.g., injection); Not determined by stud	ly authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Invertebrate	; Arthropods; Dermatophagoides farinae; A	dult		
Health Outcome:	Mortality				
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	1332803				
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.	
Domain 7: Data Present	ation and Anal	lvsis			
	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 tables.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None				
Overall Qualit	ty Deterr	nination	Medium		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Experimental and Applied Acarology 44(1):1-9. 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) Invertebrate; Arthropods; <i>Dermatophagoides farinae</i>; Adult Mortality Dibutyl phthalate (DBP) 1323180 				
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	Purity 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 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	Medium	A one-time dose was used, but few details were 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 was reported and suitable for the study type.	
	Metric 11:	Number of Exposure Groups/	Low	Four to six treatments were used, but exposure levels were not reported.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via contact to treated fabric.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from an eight-year-old lab colony.	
	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	Medium	There were 25 mites/treatment with four replicates.	
Domain 5: Outcome As	sessment				
2 smain 5. Outcome As	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.	
		C	8		
Continued on next page					

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HERO ID: 1323180 Table: 1 of 1

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Study Citation:	Kim, H. K.,	Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and				
Duration	Overall Dura	goldes pteronyssinus (Acari: Pyroglyphidae) $0 = 4 \text{ days} (0.96\text{h})$: Exposure Duration). Experimental $(0 - 4 \text{ days})$	and Applied Acarology 44(1):1-9.		
Evnosure Route	Terrestrial: N	J/Δ (e.g. injection): Not determined by stud	ly authors (i.e., c	hemical of interest in exposure water, but unable to determine exact untake route)		
Media. Path:	Terrestriar, 1	(71 (e.g., injection), Not determined by stud	<i>iy uutions</i> (<i>i.e.</i> , <i>e</i>	included of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate:	Arthropods: Dermatophagoides farinae: Ac	dult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323180	1323180				
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment				
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		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	Low	Only resultant LD50s were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This study g	This study gives dose in units of mg/m3 of felt. This may not be usable for terrestrial tox in the RE depending on exposure data available.				

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatophagoides farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031. 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) Invertebrate; Arthropods; <i>Dermatophagoides farinae</i>; Adult Mortality Dibutyl phthalate (DBP) 1341977 			
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	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.
Domain 2: Test Design				
U	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	In the Results section it is stated that "there was no mortality in the ethanol-treated con- trols."
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization	English and Such as (Test Madia	Law	
	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 Administration	Medium	Nothing in the study suggested that exposures were not 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 suitable for a dose
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response. Exposure was via substrate contact.
				•
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	Mites were kept in similar conditions to the lab colony.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 25 mites with 4-6 replicates used.
		· · · ·		
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	High	The outcome assessment methodology reported the intended outcome of interest.
Continued on next page				

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HERO ID: 1341977 Table: 1 of 1

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Study Citation:	Kim, H. K., farinae and I	Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatophagoides farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; N	N/A (e.g., injection); Not determined by stud	y authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides farinae; Ac	lult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1341977					
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	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	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the tables.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quali	Overall Quality Determination Medium					

Study Citation:	Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds						
Duration	to Dermatop	to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371.					
Duration:	Overall Duration: 0 - 4 days (0-9011); Exposure Duration: 0 - 4 days (0-9011) Terrestrial: Air N/A (a.g. injection): Not determined by study outbors (i.e. chemical of interest in exposure water, but upphle to determine exact uptake						
Exposure Koule, Modia Dath.	repute)						
Tava Spacias Agas	roule) Invertebrate: Arthropode: Dermatenhagoides faringe: Adult						
Haalth Outcomo	Mortality						
Chamical:	Montality Dibutyl phthalate (DBP)						
HERO ID:	788260	788260					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	nce						
	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%.			
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	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 2. Exposure Ch	anatanization						
Domain 5: Exposure Cr	Matria 7	Enversion and all Saustana /Tarat Madia	T				
	Metric 7:	Preparation	Low	the study provided only limited details on the measures taken to appropriately prepare test concentrations			
	Metric 8:	Consistency of Exposure	Medium	Only general methods of exposure administration were reported so assessment was			
		Administration	1.10010111	difficult to determine.			
	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 and/or exposure frequency were reported and appropriate for the study type (24h acute).			
	Metric 11:	Number of Exposure Groups/	Medium	Four to six concentrations were used, but the range was not specified.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via treated fabric.			
Domain 4: Test Organis	sm						
0	Metric 13:	Test Organism Characteristics	High	The organisms were sourced from a long-standing lab culture.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were the same for control and exposed organisms, but few details were provided.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms was reported and sufficient to characterize toxicological effects (25 organisms, replicated 4 times per treatment).			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16	Adequacy of Test Conditions	Medium	Temperature and relative humidity were reported and adequate			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
		Conti	nued on next pa	 00			
		Conti	naca on next pa				
PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

		contin	ued from previo	us page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chomical:	Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air, 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) Invertebrate; Arthropods; <i>Dermatophagoides farinae</i> ; Adult Mortality				
HERO ID:	788260				
Domain		Metric	Rating	Comments	
	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 across treatments.	
	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: Metric 22:	Statistical Methods Reporting of Data	High Low	Statistical methods were adequately described. Data for exposure-related findings were not shown for each treatment and control group, but results were described in the table as LC50 values.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None				
Overall Qualit	Overall Quality Determination Medium				

Study Citation: Duration: Exposure Route	Kang, S. W. Dermatopha Overall Dura Terrestrial: N	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 485854	Invertebrate; Arthropods; <i>Dermatophagoides pteronyssinus</i> ; Adult Mortality Dibutyl phthalate (DBP) 485854					
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	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	No mortalities were reported.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization	Evennimental System/Teat Madia	Low				
	Metric 7:	Preparation	Low	The experimental system and methods for preparation of test media were described in adequate detail. Petri dishes were used, but it was not mentioned whether they were made of glass or plastic.			
	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 was reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	Six concentrations were used.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The chemical was applied directly to cotton cloth.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Medium	Organisms were from a lab culture, but the origin of colony was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Medium	Culture and test conditions were similar.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Three replicates of 30-40 adults were used.			
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 maintenance of organism health. Temperature and relative humidity were reported.			
Continued on next page							

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Study Citation:

Duration: Exposure Route,

Media, Path: Taxa, Species, Age:

Chemical:

HERO ID:

Health Outcome:

Domain

Additional Comments:

Mortality

485854

Metric 17:

Metric 18:

Metric 19:

Metric 20:

Metric 21:

Metric 22:

Metric 23:

None

Overall Quality Determination

Domain 6: Confounding / Variable Control

Domain 7: Data Presentation and Analysis

Dibutyl phthalate (DBP)

O ID: 485854 Table: 1 of 2

Comments

There were no reported differences among the study groups in environmental conditions.

Data for exposure-related findings were not shown for each treatment and control group,

The outcome assessment methodology reported the intended outcome of interest.

There was no information in the study to suggest differences among groups.

Outcomes were assessed consistently across study groups.

Statistical methods were adequately described.

but results were described in the tables.

There were no unexpected outcomes.

Environmental Hazard Evaluation	HERO ID: 485854 Ta
continued from previous page	
Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roc Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3 Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)	ots to Dermatophagoides farinae and 3550.
Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but una	able to determine exact uptake route)

Rating

High

High

High

Medium

High

Low

High

Medium

Invertebrate; Arthropods; Dermatophagoides pteronyssinus; Adult

Outcome Assessment Methodology

Metric

Consistency of Outcome

Design and Procedures

Statistical Methods

Reporting of Data

Confounding Variables in Test

Outcomes Unrelated to Exposure

Explanation of Unexpected Outcomes

Assessment

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Study Citation:	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.						
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; A	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air; 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: Dermatophagoides pteronyssinus: Adult					
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	485854						
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	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	No mortalities were 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 Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. Petri dishes were used, but it was not mentioned whether they were made of glass or plastic.			
	Metric 8:	Consistency of Exposure Administration	Medium	Details of exposure administration were reported and exposures were administered consistently across study groups but because it was a vapor exposure, consistency is difficult to judge.			
	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/	N/A	Only one concentration was used, although both open and closed systems were evalu-			
		Spacing of Exposure Levels		ated.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The chemical was applied directly to cotton cloth, and exposure was via vapors.			
Domain 4: Test Organis	m						
C C	Metric 13:	Test Organism Characteristics	Medium	Organisms were from a lab culture, but the origin of colony was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Medium	Culture and test conditions were similar.			
	Metric 15:	Conditions Number of Organisms and	Medium	Three replicates of 30-40 adults were used.			
		Replicates per Group					
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. Temperature and relative humidity were reported.			
	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: 485854 Table: 2 of 2

	continued from previous page					
Study Citation:	Kang, S. W. Dermatopha	Kang, S. W., Kim, H. K., Lee, W. J., Ahn, Y. J. (2006). Toxicity of bisabolangelone from Ostericum koreanum roots to Dermatophagoides farinae and Dermatophagoides netrophagoides (Acari : Pyroglyphidae). Journal of Agricultural and Food Chemistry 54(10):3547-3550.				
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; Dermatophagoides pteronyssinus; Adult				
Health Outcome:	Mortality					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	485854					
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	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	ysis				
	Metric 21:	Statistical Methods	Low	Statistical methods were adequately described but results were not compared to controls; they were only compared to other chemicals.		
	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:	None					
Overall Qualit	Overall Quality Determination Medium					

Study Citation:	Kim, H. K.,	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and					
Duration: Exposure Route, Media, Path:	Dermatopha Overall Dura Terrestrial; N	Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26): /85 /- /861. Dverall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Ferrestrial; 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	Invertebrate; Arthropods; <i>Dermatophagoides pteronyssinus</i> ; Adult Mortality Dibutyd phthelate (DBP)					
Health Outcome:	Mortality						
HERO ID:	1332803	Dibutyl phthalate (DBP) 1332803					
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	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	Medium	A biological response by a control group was reported in Table 1, but it is unclear if this was the response of the control mentioned in the methods section.			
	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	A one-time dose was used, but few details were provided.			
	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	Eight concentrations over a suitable range were used.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fabric contact.			
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	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 25-30 mites with 3-5 replicates used.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16	Adequacy of Test Conditions	Medium	Control and treatment mites were held in conditions similar to rearing conditions			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.			
		Conti	nued on next pa	ge			
		Cont		o			

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HERO ID: 1332803 Table: 1 of 2

		contin	ued from previo	ous page			
Study Citation:	Kim, H. K., Dermatopha	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and Dermatophagoides nervous (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861					
Duration:	Overall Dura	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; Arthropods; Dermatophagoides pteronyssinus; Adult						
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1332803						
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	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.			
Domain 7: Data Present	ation and Anal	lysis					
	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 tables.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	Overall Quality Determination Medium						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry 52(26):7857-7861. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Air, 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) Invertebrate; Arthropods; <i>Dermatophagoides pteronyssinus</i> ; Adult						
Chemical: HERO ID:	Dibutyl phth 1332803	Mortality Dibutyl phthalate (DBP) 1332803					
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	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	No control mortalities were 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	Low	The study provided only limited details on the measures taken to appropriately prepare			
		Preparation		test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used, but few details were provided.			
	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/	High	Only one concentration was tested.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fabric contact.			
Domain 4. Test Organis	m						
2 sinum 1. 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	Medium	There were 25-35 mites with three replicates used.			
		Replicates per Group					
Domain 5: Outcome As	Matria 16:	A daguage of Test Canditions	Madium				
	Metric 17:	Auequacy of Test Conditions	Mealum Lich	Control and treatment mites were held in conditions similar to rearing conditions.			
	Matria 19	Consistency of Outcome	пign Uigh	Outcome assessment methodology addressed the intended outcome of interest.			
	Meure 18:	Assessment	підіі	Outcomes were assessed consistently across study groups.			
		A2202221110111					

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Dibutyl Phthalate

	continued from previous page				
Study Citation:	Kim, H. K.,	Kim, H. K., Tak, J. H., Ahn, Y. J. (2004). Acaricidal activity of Paeonia suffruticosa root bark-derived compounds against Dermatophagoides farinae and			
	Dermatopha	goides pteronyssinus (Acari: Pyroglyphidae). Journal of Agri	cultural and Food Chemistry 52(26):7857-7861.	
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	1: 0 - 4 days (0-96	h)	
Exposure Route,	Terrestrial; A	Air, N/A (e.g., injection); Not determined by	y study authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake	
Media, Path:	route)				
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides pteronyssin	nus; Adult		
Health Outcome:	Mortality				
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	1332803				
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.	
Domain 7: Data Present	ation and Anal	veic			
Domain 7. Data Fresent	Matria 21.	ysis Statistical Mathada	NT/A		
	Metric 21:		IN/A	Statistical analysis was not compared to control 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	High	There were no unexpected outcomes.	
Additional Comments:	closed conta	iner, method A			
Overall Quality Determination Medium					

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Experimental and Applied Acarology 44(1):1-9. 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) Invertebrate; Arthropods; <i>Dermatophagoides pteronyssinus</i>; Adult Mortality Dibutyl phthalate (DBP) 1323180 			
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	Purity or grade of the 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	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 Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used, but few details 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 exposure was reported and suitable for the study type.
	Metric 11:	Number of Exposure Groups/	Low	Four to six treatments were used, but exposure levels were not reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via contact to treated fabric.
Domain 1: Tast Organia	m			
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from an eight-year-old lab colony.
	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	Medium	There were 25 mites/treatment with four replicates used.
Domain 5: Outcom- A-	aaamart			
Domain 5: Outcome As	Matria 16	A deguage of Test Conditions	Low	Environmental conditions were not sufficiently reported to evolute if adapt-t-
	Metric 17:	Aucquacy of fest Conditions	LOW	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Meuric 17:	Outcome Assessment Methodology	пıgn	The outcome assessment methodology reported the intended outcome of interest.
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Study Citation:	Kim, H. K.,	Kim, H. K., Yun, Y. K., Ahn, Y. J. (2008). Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to Dermatophagoides farinae and				
D	Dermatopha	goides pteronyssinus (Acari: Pyroglyphidae)	. Experimental	and Applied Acarology 44(1):1-9.		
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	: 0 - 4 days (0-9	6h)		
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Not determined by study	y authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides pteronyssin	us; Adult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	1323180					
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				
	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.		
	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 i resent	Metric 21	Statistical Methods	High	Statistical methods were adequately described		
	Matria 22:	Paparting of Data	Low	Only resultant LD50s were reported		
	Metric 22.	Exploration of United Outcomes		Only resultant LD50s were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	I here were no unexpected outcomes.		
Additional Comments:	This study g	ives dose in units of mg/m3 of felt. This may	/ not be usable f	or terrestrial tox in the RE depending on exposure data available.		

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Tana Species Age:	 Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatopha farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) e, Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptakets. A reserve the product of the product						
Taxa, Species, Age: Hoolth Outcomo:	Mortality	, Anniopous, Dermaiophagolaes pieronyssi	nus; Adult				
Chemical:	tcome: Mortality Dibutyl phthalate (DBP)						
HERO ID:	1341977	1341977					
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	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.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 5:	Negative Control Response	High	In the Results section it is stated that "there was no mortality in the ethanol-treated con- trols."			
	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 Administration	Medium	Nothing in the study suggested that exposures were not 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 suitable for a dose			
		Spacing of Exposure Levels		response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via substrate contact.			
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	Mites were kept in similar conditions to the lab colony.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 25 mites with 4-6 replicates used.			
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	High	The outcome assessment methodology reported the intended outcome of interest.			
Continued on next page							

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HERO ID: 1341977 Table: 1 of 1

	continued from previous page					
Study Citation:	Kim, H. K., farinae and I	Kim, H. K., Yun, Y. K., Ahn, Y. J. (2007). Toxicity of atractylon and atractylenolide III identified in Atractylodes ovata rhizome to Dermatophagoides farinae and Dermatophagoides pteronyssinus. Journal of Agricultural and Food Chemistry 55(15):6027-6031.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	: 0 - 4 days (0-96	5h)		
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Not determined by stud	y authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Dermatophagoides pteronyssin	<i>us</i> ; Adult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1341977					
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	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	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 tables.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Quality Determination Medium						

Study Citation:	Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds						
Duration	to Dermatop	to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371.					
Evnosura Routa	Terrestrial: /	$\Lambda_{ir} N/A$ (e.g. injection): Not determined b	n. 0 - 4 uays (0-9 v study authors ((i.e., chemical of interest in exposure water, but unable to determine exact untake			
Media Path.	route)	All, IVA (e.g., injection), Not determined b	y study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake			
Tava Species Age	Invertebrate:	Arthropods: Dermatophagoides pteropyssi	nus Adult				
Health Outcome	Mortality	Tunopous, Dermaiophagotaes pieronyssi	nus, i idun				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	788260						
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	Chemical purity was reported as 99%.			
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	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 enpropriately property			
	Wieute 7.	Preparation	LOW	test concentrations			
	Metric 8:	Consistency of Exposure	Medium	Only general methods of exposure administration were reported so assessment was			
	metrie 6.	Administration	meanum	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/	Medium	Four to six concentrations were used, but a range was not specified.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via treated fabric.			
Domain 4: Test Organisi	m						
0	Metric 13:	Test Organism Characteristics	High	Organisms were sourced from a long-standing lab culture.			
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were the same for control and exposed organisms, but few			
		Conditions		details were provided.			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms was reported and sufficient to characterize toxicological			
		Replicates per Group		effects (25 organisms, replicated 4 times per treatment).			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Temperature and relative humidity were reported and adequate.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
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		contin	ued from previo	ous page			
Study Citation: Duration:	Wang, Z., K to Dermatop Overall Dura	Wang, Z., Kim, H. K., Tao, W., Wang, M., Ahn, Y. J. (2011). Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to Dermatophagoides farinae and Dermatophagoides pteronyssinus (Acari: Pyroglyphidae). Journal of Medical Entomology 48(2):366-371. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route, Modio Dothy	Terrestrial; Air, N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake						
Tava Species Age	Iouic) Invertebrate:	route)					
Health Outcome	Mortality	Anthopous, Dermalophagolaes pieronyssin	ius, Adun				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	788260						
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	Low	The study did not provide enough information to allow a comparison of environmental conditions across treatments.			
	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	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the table as LC50 values.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Misra, S., Si	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and					
Duration: Exposure Route, Madia Bath	understandin Overall Dura Terrestrial; F	g of xenobiotic-mediated male reproductivation: 0 - 4 days (0-96h); Exposure Duratio Good/Diet; Dietary	ve adversities on: 0 - 4 days	. Toxicological Sciences 141(1):278-291. (0-96h)			
Tava Species Age	Invertebrate	Arthropods: Drosophila melanogastar: A	dult				
Health Outcome	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2510760						
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 was not reported.			
Domain 2: Test Design							
e	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	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 study provided only limited details on the measures taken to appropriately prepare test concentrations although the test system was described in adequate detail. Glass vials were used			
	Metric 8:	Consistency of Exposure	Medium	One time dose to the food, few other details provided			
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10.	Concentration Exposure Duration and Frequency	Low	It was unclear what the exposure duration was, it was based on eclosion time			
	Metric 11:	Number of Exposure Groups/	Low	No information is provided on the number of exposure groups and spacing of exposure			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	levels although the range was 10 mM to 2 M 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	Low	The study did not report whether test organisms were acclimatized			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms (100) was adequate.			
Domain 5: Outcome Ass	Sessment	A degree of Test Car 1:4:	T				
	Matric 17:	Adequacy of lest Conditions	LOW	Environmental conditions were not sufficiently reported to evaluate if adequate			
	Metric 17:	Outcome Assessment Methodology	Mealum	i ne outcome assessment methodology was not reported			
		Cont	inued on nex	ct page			

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	continued from previous page						
Study Citation:	Misra, S., S	ingh, A., Ch, R., Sharma, V., Mudiam, R.	, M.K., Ran	n, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and			
	understandir	inderstanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)			
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; Ac	lult				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2510760						
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 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 Present	ation and Anal	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 text.			
	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:	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Dibutyl phth 2510760	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Reproductive/Teratogenic Dibutyl phthalate (DBP) 2510760					
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 the test substance was not reported.			
Domain 2: Test Design							
2 onian 21 1000 2 001gh	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 reported and reasonable.			
	Metric 6:	Randomized Allocation	Medium	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 although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used to the food, but few other details were provided.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Concentration Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, but it was based on eclosion time $(>10 \text{ days})$.			
	Metric 11:	Number of Exposure Groups/	Medium	There were two exposure groups that were evenly spaced.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
Domain 4: Test Organis	m						
Domain 4. Test Organisi	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 test organisms (100) was adequate.			
		Replicates per Group					
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	Medium	The outcome assessment methodology was not reported.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.			
		Conti	nued on next pa	ge			

HERO ID: 2510760 Table: 2 of 2

Study Citation:	Misra, S., Si	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and				
Duration:	Overall Dura	ition: 0 - 4 days (0-96h): Exposure Duration	: 0 - 4 days (0-9)	(1000) (1000)		
Exposure Route,	Terrestrial; F	Good/Diet; Dietary)		
Media, Path:	,					
Taxa, Species, Age:	Invertebrate;	Invertebrate: Arthropods: Drosophila melanogaster: Embryo				
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Reproductive/Teratogenic					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	2510760					
Domain		Metric	Rating	Comments		
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	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	Unexpected outcomes were satisfactorily explained		

Overall Quality Determination

Medium

Study Citation:	Misra, S., S	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xanohiotic mediated male reproductive adversities. Toxicological Sciences 141(1):278-201					
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; F	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Invertebrate	Invertebrate; Arthropods; Drosophila melanogaster; Embryo					
Health Outcome:	Reproductiv	Reproductive/Teratogenic					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	2510760						
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 Pufity	Low	Purity or grade of the test substance was not reported.			
Domain 2: Test Design							
6	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 reported and reasonable.			
	Metric 6:	Randomized Allocation	Medium	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization	Environmental Contains //Tait Madia	Madian				
	Metric 7:	Preparation	Medium	test concentrations although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used to the food, but few other details were provided.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, but it was based on eclosion time $(>10 \text{ days})$.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two exposure groups were used and they were adequately spaced apart.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
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 Replicates per Group	Medium	The number of test organisms (100) was adequate.			
		,					
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	Medium	The outcome assessment methodology was not reported.			
		Conti	inued on next pa	ge			

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		contin	ued from previo	bus page		
Study Citation:	Misra, S., S understandir	Aisra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and inderstanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.				
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10) days	č ()		
Exposure Route,	Terrestrial; F	Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Drosophila melanogaster; Embryo				
Health Outcome:	Reproductiv	Reproductive/Teratogenic				
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	2510760					
Domain		Metric	Rating	Comments		
	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.		
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 shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	Multiple stu	dies were reported, which led to a lack of cla	rity regarding te	st set up and conditions.		
Overall Qualit	ty Detern	nination	Medium			

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Study Citation:	Williams, M Conserved In Overall Dura	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321. Overall Duration: 4 - 10 days: Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:	Invertebrate: Arthropoda: Dresophila malanegater: CSOBC wild type leb strain: Embryo						
Taxa, Species, Age: Health Outcome:	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild type lab strain; Embryo Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	3350270						
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 was not reported			
Domain 2: Test Design							
C C	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, control response was set to 100%			
	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 experimental system and/or test media preparation methods were adequately re-			
	Wette 7.	Preparation	Wiedium	ported but there is some question as to the stability of DBP in food over time			
	Metric 8:	Consistency of Exposure	Medium	Some details of exposure administration were reported and exposures seemed to be			
		Administration		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 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			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet			
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	Medium	10 replicates is adequate			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
		Conti	inued on next pa	ge			

Environmental Hazard Evaluation

HERO ID: 3350270 Table: 1 of 5

	continued from previous page						
Study Citation:	Williams, M Conserved I	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10	0 days				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild type lab strain; Embryo						
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	3350270						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	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	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:	The outcome	e was expression of metabolic and xenobioti	c-related genes.				
Overall Quali	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route, Madia Path:	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Behavioral Dibutyl phth 3350270	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild type lab strain; Embryo Behavioral Dibutyl phthalate (DBP) 3350270					
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 was not reported			
Domain 2: Test Design							
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 organisms were allocated to study groups.			
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 there is some question as to the stability of DBP in food over time			
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of exposure administration were reported and exposures seemed to be 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 and/or exposure frequency were reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	Medium	Three concentrations and a control were sufficient to elicit a response			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet			
Domain 4: Test Organis	m						
_ sham 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	High	all pretreatment conditions were the same for control and exposed organisms			
	Metric 15:	Conditions Number of Organisms and	Medium	20 flies replicated 10 times is adequate			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not 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: 3350270 Table: 2 of 5

		contin	ued from previo	us page			
Study Citation:	Williams, M Conserved I	'illiams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily onserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.					
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;	Arthropods; Drosophila melanogaster; CSO	ORC wild type la	b strain; Embryo			
Health Outcome:	Behavioral						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	3350270						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups			
		Assessment					
Domain 6: Confounding	Variable Co	atrol					
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 7. Data Present	ation and Anal	vsis					
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	unexpected outcomes were satisfactorily explained			
		L	Ø	1			
Additional Comments:	locomotor, s	leep, Fig 4, feeding behavior					
Overall Quali	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route, Media, Path:	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolution Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Dibutyl phth 3350270	nvertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild type lab strain; Adult Aechanistic-Biomarkers (exposure and effect)-Cell signaling/function Dibutyl phthalate (DBP) 350270					
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 was not reported			
Domain 2. Test Design							
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, control response was set to 100%			
	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/or test media preparation methods were adequately re- ported but there is some question as to the stability of DBP in food over time			
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of exposure administration were reported and exposures seemed to be 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 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			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet			
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	all pretreatment conditions were the same for control and exposed organisms			
	Metric 15:	Conditions Number of Organisms and	Medium	10 replicates is adequate			
		Replicates per Group					
Domain 5: Outcome Ac	recement						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	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: 3350270 Table: 3 of 5

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Study Citation:	Williams, M Conserved I	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.				
Duration:	Overall Dura	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; CSO	ORC wild type l	ab strain; Adult		
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell signa	aling/function			
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3350270					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups		
		Assessment				
Domain 6: Confounding	r / 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		
	metile 19.	Design and Procedures	2011	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	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 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained		
		r ····································	-8	· · · · · · · · · · · · · · · · · · ·		
Additional Comments:	The outcome	e was expression of metabolic and xenobiotic	c-related genes.			
Overall Qualit	v Detern	nination	Medium			

Study Citation: Duration: Exposure Route, Media, Path:	Williams, M Conserved In Overall Dura Terrestrial; F	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Nutritional & Dibutyl phth 3350270	nvertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild type lab strain; Embryo Nutritional & Metabolic Dibutyl phthalate (DBP) 3350270					
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	Purity or grade of the test substance was not reported.			
Domain 2: Test Design							
2 onian 21 Test 2 toigh	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 organisms were allocated to study groups.			
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 there is some question as to the stability of DBP in food over time.			
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of exposure administration were reported and exposures seemed to be 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 and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Three concentrations and a control were sufficient to elicit a response.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
Domain 4: Test Organis							
_ 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	High	All pretreatment conditions were the same for control and exposed organisms.			
	Metric 15:	Conditions Number of Organisms and	Medium	Twenty flies with ten replicates were used.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels were not reported.			
	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: 3350270 Table: 4 of 5

		contin	ued from previo	us page			
Study Citation:	Williams, M Conserved I	'illiams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily onserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.					
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10	0 days				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; CSORC wild type lab strain; Embryo						
Health Outcome:	Nutritional & Metabolic						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	3350270						
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			
	incure 19.	Design and Procedures	2011	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	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:	Glucose, gly	cogen, trehalose, lipid content					
Overall Qualit	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route, Madia Path:	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 3350270	nvertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild type lab strain; Embryo Aortality Dibutyl phthalate (DBP) 350270					
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 was 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, control response was set to 100%			
	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/or test media preparation methods were adequately re- ported but there is some question as to the stability of DBP in food over time			
	Metric 8:	Consistency of Exposure Administration	Medium	Some details of exposure administration were reported and exposures seemed to be 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/or exposure frequency were reported and appropriate for the study type			
	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	Exposure was via diet			
Domain 4: Test Organis	m						
sector organio	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	10 replicates is adequate			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported			
	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: 3350270 Table: 5 of 5

continued from previous page						
Study Citation:	Williams, M Conserved I	Williams, M. J., Wiemerslage, L., Gohel, P., Kheder, S., Kothegala, L. V., Schioth, H. B. (2016). Dibutyl Phthalate Exposure Disrupts Evolutionarily Conserved Insulin and Glucagon-Like Signaling in Drosophila Males. Endocrinology 157(6):2309-2321.				
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;	Arthropods; Drosophila melanogaster; CS	ORC wild type la	b strain; Embryo		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3350270					
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	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	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	unexpected outcomes were satisfactorily explained		
Additional Comments:	Mortality Fig	g 1				
Overall Quality Determination Medium						

Study Citation: Duration: Exposure Route,	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Media, Path:	Invertebrate: Arthropods: Drosonhila melanogaster: Embryo						
Taxa, Species, Age: Health Outcome:	Reproductiv	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Embryo Reproductive/Teratogenic					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2510760						
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 was not reported.			
Domain 2: Test Design							
C C	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 reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	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	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure	Medium	One time dose to the food, few other details provided			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, it was based on eclosion time (>10 days)			
	Metric 11:	Number of Exposure Groups/	Medium	Two exposure groups, suitably spaced			
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via diet			
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			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The number of test organisms (100) was adequate.			
		_					
Domain 5: Outcome As	Matria 16	A dequeey of Test Canditions	Law	Environmental conditions were not sufficiently were stadied over the terminate if a large t			
	Metric 17:	Aucquacy of rest Conditions Outcome Assessment Methodology	LOW Medium	Environmental conditions were not sufficiently reported to evaluate if adequate The outcome assessment methodology was not reported			
	within 17.		iviculuili	The outcome assessment memorology was not reported			
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	continued from previous page					
Study Citation:	Misra, S., S	ingh, A., Ch, R., Sharma, V., Mudiam, R.,	M.K., Ram, K.	R. (2014). Identification of Drosophila-based endpoints for the assessment and		
Duration	understandin	understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.				
Duration: Euroguno Douto	Tormostrial, I	Overal Duration. 11 - 21 days, Exposure Duration. 11 - 21 days				
Exposure Koule, Modio Both	Terrestriar; r	Terrestriar, Food/Diet, Dietary				
Tava Spacias Agos	Invariabrata	Investe Anthere de Deservicie antes Enderer				
Taxa, Species, Age:	Domes du stiv	Alunopous, Drosophila metanogaster, Enn	biyo			
Chamical	Dibutul abth	e/ reratogenic				
UEDO ID.	2510760	larate (DBP)				
HERO ID:	2510760					
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 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	N 11	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	vicio.				
Domain 7. Data Fresent	Motrio 21:	ysis Statistical Matheda	High	Statistical mathe do more a domataly described		
	Metric 21: Matria 22:	Statistical Methods Deporting of Data	High High	Statistical methods were adequately described		
	Metric 22:	Explanation of Unavasted Outcomes	High High	Data for exposure-related findings were snown for each treatment and control group		
	wieute 25:	Explanation of Onexpected Outcomes	пign	unexpected outcomes were sausractority explained.		
Additional Comments	Multiple stu	dies reported this led to a lack of clarity regu	arding test set ur	and conditions		
raditional comments.	muniple stu	ales reported, this led to a lack of clarity lega	aroning test set up			
Overall Onels	ty Dotor	nination	Modium			
	Overall Quality Determination Medium					

Study Citation: Duration: Exposure Route, Media, Path:	Misra, S., Si understandin Overall Dura Terrestrial; F	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary					
Taxa, Species, Age: Health Outcome:	Invertebrate; ADME (biot	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Embryo ADME (biotransformation)					
Chemical: HERO ID:	Dibutyl phth 2510760	Dibutyl phthalate (DBP) 2510760					
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 the test substance was not reported.			
Domain 2: Test Design							
e	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 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	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure Administration	Medium	A one-time dose was used to the food, but few other details were provided.			
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were not measured, but body burdens were.			
	Metric 10:	Exposure Duration and Frequency	Low	The exact duration was not clear. Eggs were exposed until eclosion, and the text did state that flies were three days old when assessed. The total duration was reported as 12-13 days.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one level was tested.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
Domain 4. Test Organis							
Domain 1. 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	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	High	The outcome assessment methodology addressed the intended outcome of interest.			
	Continued on next page						

continued from previous page							
Study Citation:	Misra, S., S understandir	lisra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and inderstanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; F	Terrestrial; Food/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; Em	bryo				
Health Outcome:	ADME (biot	ransformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2510760						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- 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	N/A	Statistical analysis is not typical for this type of outcome.			
	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:	None						
Overall Qualit	ty Detern	nination	Medium				

Study Citation: Duration: Exposure Route,	Misra, S., Si understandin Overall Dura Terrestrial; F	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary				
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Reproductive Dibutyl phth 2510760	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP) 2510760				
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	Low	Purity or grade of test substance was not reported.		
Domain 2: Test Design						
Domain 2. Test Design	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 reported and reasonable		
	Metric 6:	Randomized Allocation	Medium	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 although the test system was described in adequate detail. Glass vials were used.		
	Metric 8:	Consistency of Exposure	Medium	One time dose to the food, few other details provided		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, it was based on eclosion time (>10 days)		
	Metric 11:	Number of Exposure Groups/	Medium	Two exposure groups, suitably spaced		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via diet		
Domain 4. Test Organis	m					
Domain 7. 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	Medium	The number of test organisms (100) was adequate.		
		Replicates per Group				
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	Medium	The outcome assessment methodology was not 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						

Page 720 of 983
		contin	ued from previ	ous page			
Study Citation:	Misra, S., S	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and					
Demotions	understandir	understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days				
Exposure Route,	Terrestrial; F	ienestriai, rood/Diet, Dietary					
Media, Path:	Investe hander Antherine der Deussen bille und eine eine Andelte						
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Adult						
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	2510760						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	y / 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			
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	unexpected outcomes were satisfactorily explained.			
	Multiple studies reported, this led to a lack of clarity regarding test set up and conditions						

Study Citation: Duration: Exposure Route, Modia Path:	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assess understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Developmen Dibutyl phth 2510760	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 2510760					
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 was not reported.			
Domain 2: Test Design							
Domain 21 Teor Design	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 reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	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 although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure	Medium	One time dose to the food, few other details provided			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, it was based on eclosion time (>10 days)			
	Metric 11:	Number of Exposure Groups/	Medium	Two exposure groups, suitably spaced			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	exposure was via diet			
Domain 1. Test Organia	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	Low	The study did not report whether test organisms were acclimatized			
	Metric 15:	Conditions Number of Organisms and	Medium	The number of test organisms (100) was adequate.			
		Replicates per Group					
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	Medium	The outcome assessment methodology was not 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							

		contin	ued from previ	ous page			
Study Citation:	Misra, S., S	lisra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and					
Dermetterne	understandir	understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.					
Duration:	Overall Dura	Terrestrial: Food/Dist: Distany					
Exposure Route,	Terrestrial; F	ood/Diet; Dietary					
Media, Path:							
Taxa, Species, Age:	Invertebrate; Arthropods; Drosophila melanogaster; Embryo						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2510760						
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			
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	unexpected outcomes were satisfactorily explained.			
	Multiple studies reported, this led to a lack of clarity regarding test set up and conditions						

Study Citation:	Misra, S., Si	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and				
Duration: Exposure Route, Modia Patha	understandin Overall Dura Terrestrial; F	ng of xenobiotic-mediated male reproduction ation: Not-reported; Exposure Duration: N Food/Diet; Dietary	ve adversities lot-reported	. Toxicological Sciences 141(1):278-291.		
Media, Path: Taya Species Age:	Invertebrate	Arthropods: Drosophila malanogastar: Fi	mbryo			
Health Outcome	Mortality	Anthropods, Drosophila metanogaster, En	moryo			
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2510760					
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	Low	Purity or grade of test substance was not reported.		
Domain 2: Test Design						
U	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	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
I IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations although the test system was described in adequate detail. glass vials were used		
	Metric 8:	Consistency of Exposure	Medium	One time dose to the food, few other details provided		
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	High	72 hour exposure duration		
	Metric 11:	Number of Exposure Groups/	Low	No information is provided on the number of exposure groups and spacing of exposure		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	levels although the range was 10 mM to 2 M 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	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.		
		Replicates per Group				
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 reported		
		Cont	inued on nex	xt page		
				• •		

	continued from previous page					
Study Citation:	Misra, S., S	ingh, A., Ch, R., Sharma, V., Mudiam, R.	, M.K., Ran	n, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and		
	understandir	ng of xenobiotic-mediated male reproductiv	e adversities	. Toxicological Sciences 141(1):278-291.		
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: No	ot-reported			
Exposure Route,	Terrestrial; F	Food/Diet; Dietary				
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; Drosophila melanogaster; En	nbryo			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2510760					
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 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		
Domain 7: Data Present	ation 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	Low	The study did not report any measures of variability		
Additional Comments:	None					
Overall Quality Determination Lo			Low			

Study Citation: Duration: Exposure Route, Media Path:	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mechanistic- Dibutyl phth 2510760	nvertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Reproductive/Teratogenic Dibutyl phthalate (DBP) 2510760					
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 the test substance were not reported.			
Domain 2: Test Design							
2 oniuni 21 Test 2 teign	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 reported and reasonable.			
	Metric 6:	Randomized Allocation	Medium	Researchers did not report how organisms were allocated to study groups.			
Damain 2: Error or Ch							
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Madia	Madium				
	Metric 7:	Preparation	Medium	test concentrations although the test system was described in adequate detail. Glass vials were used.			
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used to the food, but few other details were provided.			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	Low	It was unclear what the exact exposure duration was, but it was based on eclosion time $(>10 \text{ days})$.			
	Metric 11:	Number of Exposure Groups/	Medium	There were two exposure groups that were evenly spaced.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via diet.			
		· · ·					
Domain 4: Test Organisi	m Matri: 12	Test Organism Charles in the	TT: 1				
	Matria 14:	A a climatization and Protectment	High	The study did not expert whether test experiment were obtained from a reliable source.			
	Metric 14:	Conditions	Low	The study did not report whether test organisms were acclimatized.			
	wieute 15:	Replicates per Group	Wiedfulli	The number of test organisms (100) was adequate.			
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	Medium	The outcome assessment methodology was not reported.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.			
		Contin	ued on next pa	ge			

HERO ID: 2510760 Table: 2 of 2

		contin	ued from previ	ous page			
Study Citation:	Misra, S., S	Misra, S., Singh, A., Ch, R., Sharma, V., Mudiam, R., M.K., Ram, K. R. (2014). Identification of Drosophila-based endpoints for the assessment and					
	understandir	understanding of xenobiotic-mediated male reproductive adversities. Toxicological Sciences 141(1):278-291.					
Duration:	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; Adult						
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell signation	aling/function-R	eproductive/Teratogenic			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2510760						
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.			
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	Unexpected outcomes were satisfactorily explained.			
	Multiple studies were reported, which led to a lack of clarity regarding test set up and conditions.						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Du, L., Li, G Environmen Overall Dura Terrestrial; S Invertebrate; Mortality Dibutyl phth 2816887	A, Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Bital Science and Pollution Research 22(6):46 tition: 0 - 4 days (0-96h); Exposure Duration Bediment; Not determined by study authors (Worms (e.g., Annelids, Nematodes); <i>Eisent</i> alate (DBP)	iomarker respons 60-4669. 1: 0 - 4 days (0-9 i.e., chemical of <i>ia fetida</i> ; Adult	ses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. 6h) interest in exposure water, but unable to determine exact uptake route)
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 DBP was reported to be Shandong Jingbo Agricultural Chemical Co. Ltd. It was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 98%.
Domain 2: Test Design	Metric 4:	Negative Controls	Low	A control group is mentioned, but results are not shown and it is not reported whether it is a true negative control or a vehicle/solvent control.
	Metric 5:	Negative Control Response	Low	The negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into study groups.
Domain 3: Exposure Ch	aracterization		-	
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test media. The filter paper test was used for this portion of the study. Glass culture dishes were used and washed in chromic acid, and dried at 300C overnight.
	Metric 8:	Consistency of Exposure Administration	Low	All exposures were for 48h at 20C with a 12L:12D photoperiod, but little other informa- tion was provided to determine consistency.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was 48h, which is typical of an acute toxicity test.
	Metric 11:	Number of Exposure Groups/	High	There were seven exposure levels and spacing appeared adequate for a response.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via "soil," which was filter paper in this case.
Domain 4: Test Organis	m			
C C	Metric 13:	Test Organism Characteristics	High	The worms were from the Shandong Agricultural University and were reported to be adults.
	Metric 14:	Acclimatization and Pretreatment	High	A 24h depuration period was provided in the same conditions as the test (Petri dish lined with filter paper at 20C and 12/12h photoperiod).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten organisms per test chamber and there were three replicates per treatment level.

Domain 5: Outcome Assessment

Continued on next page ...

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

		conti	nued from previo	ous page			
Study Citation:	Du, L., Li, G	Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h): Exposure Duration	on: 0 - 4 days (0-9	6h)			
Exposure Route.	Terrestrial: S	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: Worms (e.g., Annelids, Nematodes): <i>Eisenia fetida</i> : Adult					
Health Outcome:	Mortality		, j,				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2816887						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-LC50 values.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Worms were assessed for mortalities every 24h.			
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.			
Domain 7: Data Presen	tation and Anal	vsis					

mum /. Dutu I 1050	mation and I mai	5515		
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section of the paper.
	Metric 22:	Reporting of Data	Low	Only LC50 values were reported in the text. No mortality or control data was reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. 95% confidence intervals
				were reported in the text.

Additional Comments: This portion of the evaluation was on the 24 and 48h acute toxicity of DBP on E. fetida using the filter paper test. Mortality was selected as the outcome of interest.

Overall Quality Determination

Medium

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. Overall Duration: 0 - 4 days (0.96b): Exposure Duration: 0 - 4 days (0.96b).					
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Not determined by stud	dy authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	- ·						
Taxa, Species, Age:	Invertebrate;	; Arthropods; <i>Eisenia fetida</i> ; Savigny; Adult	t				
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	3625226	3625226					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		TT: 1				
	Metric 1:	Test Substance Identity	High	Test substance was listed by name and CAS number.			
	Metric 2:	Test Substance Source	High	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							
C	Metric 4:	Negative Controls	High	Controls were utilized.			
	Metric 5:	Negative Control Response	Low	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 is unclear whether DBP was dissolved in a solvent prior to addition to filter paper.			
	Metric 8:	Consistency of Exposure Administration	Medium	It is unclear if solvent was utilized and if 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/	Low	Exposure concentrations were not stated. At least five concentrations were utilized for			
		Spacing of Exposure Levels		the definitive test (exact number of exposure groups not specified); unclear whether one of these concentrations is a control (blank).			
	Metric 12:	Testing at or Below Solubility Limit	Low	Test substance was applied to filter paper using water or other solvent. Unclear which was used and if concentrations exceeded solubility limit.			
Domain 4: Test Organis	m						
6	Metric 13:	Test Organism Characteristics	Low	Source of the test organisms not stated.			
	Metric 14:	Acclimatization and Pretreatment	Low	An acclimation process/procedure was not reported.			
	Metric 15:	Conditions Number of Organisms and	Low	There were ten replicate worms per test concentration (one worm per vial).			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	It was unclear whether worms had adequate air flow during the exposure period.			
		Conti	nued on next pa	ge			

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Dibutyl Phthalate

		contin	ued from previ	ous page		
Study Citation:	Neuhauser, l	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; Arthropods; Eisenia fetida; Savigny; Adult					
Health Outcome:	Mortality					
Chemical:	Dibutyl phthalate (DBP)					
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	High	Mortality was assessed in study groups after the 48 hour exposure duration.		
		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	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 Present	ation and Anal	vsis				
Domain / Dua Present	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	The LC50 value with CI was 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	he artificial soil test.		
Overall Quali	ty Detern	nination	Medium			

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Study Citation: Duration: Exposure Route.	Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)								
Media. Path:	Terrestria, e	renestral, beament, not determined by study address (ne., enclined of interest in exposure water, but anable to determine exact uptake foure)							
Taxa, Species, Age:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Eiser	<i>ia fetida</i> ; Adult						
Health Outcome:	Mortality								
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	2816887								
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 DBP was reported to be Shandong Jingbo Agricultural Chemical Co. Ltd. It was not reported if it was analytically verified.					
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 98%.					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	Study authors reported that a concurrent negative control was used for this part of the study.					
	Metric 5:	Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported.					
	Metric 6:	Randomized Allocation	Medium	The worms were reported to be randomly allocated to treatment groups for this portion of the study.					
Domain 3: Exposure Ch	aracterization								
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	Limited details were provided on the preparation of the test media for each concentra-					
		Preparation		tion. Artificial soil was used as the substrate/test media for this test. Glassware was used and washed in chromic acid, then dried at 300C overnight.					
	Metric 8:	Consistency of Exposure Administration	Low	All exposures took place in 1000mL containers with 500g of treated soil with 10 worms each. Experiments were conducted at 20C with a 12L:12D photoperiod. All containers were sealed with plastic film.					
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.					
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be 14d. This is longer than is typical for an acute toxicity test, but it was effective to obtain results pertinent to the outcome of interest.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure levels and spacing appeared adequate for a response.					
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via artificial soil.					
Domain 4: Test Organis	m								
6.4.4	Metric 13:	Test Organism Characteristics	High	The worms were from the Shandong Agricultural University and were reported to be adults.					
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Worms were acclimated for 24h in similar conditions to those in the study (glass Petri dishes lined with filter paper at a 12/12h photoperiod and 20C).					
		Conti	inued on next pa	ge					

Environmental Hazard Evaluation

HERO ID: 2816887 Table: 1 of 1

		contin	ued from previ	ous page		
Study Citation:	Du, L., Li, G Environment	Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669.				
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Temperateicle Sediments Net determined by study outbors (i.e., chemical of interest in synappyre water, but unchie to determine event untake route)					
Exposure Koute,	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Tava Spacias Agai	Invertebrate: Worms (e.g. Annelids Nematodes): Fisenia fatida: Adult					
Health Outcome	Mortality	, worms (e.g., Annends, Nenhalodes), Eisenh	<i>a jenaa</i> , Auun			
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	2816887					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten organisms per test chamber and there were three replicates per treatmen level.		
Domain 5: Outcome A	ssessment					
Johnani J. Outcome A	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reporte cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is unclear what they were fed for the duration of this study.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–LC50 values.		
	Metric 18:	Consistency of Outcome Assessment	High	Details regarding the execution of the study protocol for outcome assessment were lim- ited. Worms were assessed for mortality at 14d, but it is unclear if they were assessed a any other point.		
Domain 6: Confoundin	og / 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 Presen	ntation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section of the paper.		
	Metric 22:	Reporting of Data	Low	Only LC50 values were reported in the text. No mortality or control data was reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. 95% confidence intervals were reported in the text.		

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Du, L., Li, G., Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Biomarker responses in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. Environmental Science and Pollution Research 22(6):4660-4669. 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) Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Eisenia fetida</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology) Dibutyl phthalate (DBP)						
Demain	2810887	M-+	Datina	Commente			
Domain Domain 1: Test Substan	ce	Metric	Kating	Comments			
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Shandong Jingbo Agricultural Chemical Co. Ltd. It was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 98%.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported that a concurrent negative control was used for this part of the			
	Metric 5:	Negative Control Response	High	study.			
	Metric 5.	Regative Control Response	Ingn	outcomes of interest.			
	Metric 6:	Randomized Allocation	Medium	The worms were reported to be randomly allocated to treatment groups for this portion of the study.			
Domain 3: Exposure Ch	aracterization						
Domain C. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test media for each concen- tration. Artificial soil was used as the substrate/test media for this test. Glassware was washed in chromic acid and dried at 300C overnight.			
	Metric 8:	Consistency of Exposure Administration	Low	All exposures took place in 1000mL containers with 500g of treated soil with 10 worms each. Experiments were conducted at 20C with a 12L:12D photoperiod. All containers were sealed with plastic film.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 28d. This was adequate for the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/	High	There were five exposure levels and spacing appeared adequate for a response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via artificial soil.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	The worms were from the Shandong Agricultural University and were reported to be adults.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation was for 24h in glass Petri dishes at 20C with a 12/12h photoperiod, which was similar to the study conditions.			
	Continued on next page						

Environmental Hazard Evaluation

HERO ID: 2816887 Table: 1 of 1

Study Citation: Du, L Enviro Enviro Duration: Overa Exposure Route, Terres Media, Path: Taxa, Species, Age: Invert Taxa, Species, Age: Invert Health Outcome: Mech. Chemical: Dibut, HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Metric Domain 6: Confounding / Varial Metric Metric	, Li, G., nmental l Durati trial; Soi ebrate; W nistic-B l phthal 87 15: 16: 17:	Liu, M., Li, Y., Yin, S., Zhao, J. (2015). Bi I Science and Pollution Research 22(6):460 on: > 21 days; Exposure Duration: > 21 o il; Not determined by study authors (i.e., c Vorms (e.g., Annelids, Nematodes); <i>Eiseni</i> Siomarkers (exposure and effect)-Oxidative ate (DBP) Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	iomarker respon 60-4669. days hemical of inter <i>ia fetida</i> ; Adult e stress (includir Rating Medium Medium	Ises in earthworms (Eisenia fetida) to soils contaminated with di-n-butyl phthalates. rest in exposure water, but unable to determine exact uptake route) ng redox biology) Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is reported. Worms were allowed to empty their guts prior to the start of the test. It is
Duration: Overa Exposure Route, Terres Media, Path: Terres Taxa, Species, Age: Invert Health Outcome: Mech. Chemical: Dibuty HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Metric Metric Domain 6: Confounding / Varial Metric	l Durati trial; Soi ebrate; W nistic-B l phthal 87 15: 16: 17:	on: > 21 days; Exposure Duration: > 21 d il; Not determined by study authors (i.e., c Vorms (e.g., Annelids, Nematodes); <i>Eiseni</i> tiomarkers (exposure and effect)-Oxidative ate (DBP) <u>Metric</u> Number of Organisms and <u>Replicates per Group</u> Adequacy of Test Conditions	days hemical of inter <i>ia fetida</i> ; Adult e stress (includir <u>Rating</u> Medium Medium	rest in exposure water, but unable to determine exact uptake route) ng redox biology) Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is reported. Worms were allowed to dentify of the dentity of the start of the test. It is
Exposure Route, Terres Media, Path: Terres Taxa, Species, Age: Invert Health Outcome: Mech. Chemical: Dibut HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Domain 5: Outcome Assessmen Metric Domain 6: Confounding / Varial Metric	rial; Soi brate; W nistic-B l phthal: 87 15: 16: 17:	il; Not determined by study authors (i.e., c Vorms (e.g., Annelids, Nematodes); <i>Eiseni</i> Giomarkers (exposure and effect)-Oxidative ate (DBP) <u>Metric</u> Number of Organisms and <u>Replicates per Group</u> Adequacy of Test Conditions	hemical of inter <i>ia fetida</i> ; Adult e stress (includir <u>Rating</u> Medium Medium	rest in exposure water, but unable to determine exact uptake route) ng redox biology) Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is reported. Worms were allowed to empty their guts prior to the start of the test. It is
Media, Path: Taxa, Species, Age: Invert Health Outcome: Mech. Chemical: Dibut, HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Domain 6: Confounding / Varial Metric	brate; W nistic-B 1 phthal 87 15: 16: 17:	Vorms (e.g., Annelids, Nematodes); <i>Eiseni</i> Fiomarkers (exposure and effect)-Oxidative ate (DBP) Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	<i>a fetida</i> ; Adult e stress (includin <u>Rating</u> Medium Medium	Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is made and the dearthing of the interturb.
Taxa, Species, Age: Invert Health Outcome: Mech. Chemical: Dibuty HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Metric Metric Domain 6: Confounding / Varial Metric Metric	brate; W nistic-B 1 phthal 87 15: 16: 17:	Vorms (e.g., Annelids, Nematodes); <i>Eiseni</i> Fiomarkers (exposure and effect)-Oxidative ate (DBP) Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	<i>a fetida</i> ; Adult e stress (includin Rating Medium Medium	Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is modement of a for the destribute of the interture.
Health Outcome: Mech. Chemical: Dibut. HERO ID: 28168 Domain Metric Domain 5: Outcome Assessmen Metric Metric Metric Domain 6: Confounding / Varial Metric	nistic-B 1 phthal 87 15: 16: 17:	Giomarkers (exposure and effect)-Oxidative ate (DBP) Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	Rating Medium Medium	Comments Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is reported was a substrate duration of the start of the test.
Chemical: Dibut HERO ID: 28168 Domain Domain 5: Outcome Assessmen Metric Metric Domain 6: Confounding / Varial Metric	1 phthal. 87 15: 16: 17:	Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	Rating Medium Medium	Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is much expected by for the start of the interview.
Domain 5: Outcome Assessmen Metric Domain 5: Outcome Assessmen Metric Domain 6: Confounding / Varial Metric	15: 16: 17:	Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	Rating Medium Medium	Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is reported. Worms were allowed to empty their guts prior to the start of the test. It is
Domain Metric Domain 5: Outcome Assessmen Metric Metric Domain 6: Confounding / Varial Metric	15: 16: 17:	Metric Number of Organisms and Replicates per Group Adequacy of Test Conditions	Rating Medium Medium	Comments There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is
Metric Domain 5: Outcome Assessmen Metric Metric Domain 6: Confounding / Varial Metric	15: 16: 17:	Number of Organisms and Replicates per Group Adequacy of Test Conditions	Medium Medium	There were ten organisms per test chamber and there were three replicates per treatment level. Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is
Domain 5: Outcome Assessmen Metric Metric Domain 6: Confounding / Varial Metric	16:	Adequacy of Test Conditions	Medium	Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is made and the dust it is a dust it is a dust it is a dust it is a dust it.
Metrie Metrie Metrie Domain 6: Confounding / Varial Metrie	16: 17:	Adequacy of Test Conditions	Medium	Organisms were reported to be kept at 20C with a 12L:12D photoperiod. It was reported cow dung was used as a substrate and as the food source, but how much was used was not reported. Worms were allowed to empty their guts prior to the start of the test. It is
Metrie Metrie Domain 6: Confounding / Varial Metrie	17:			unclear what they were led for the duration of this study.
Metrie Domain 6: Confounding / Varial Metrie		Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–SOD, CAT, POD, GST, GSH, MDA.
Domain 6: Confounding / Varial 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 sampled at days 7, 14, 21, and 28 and assessed for SOD, CAT, POD, GST, GSH, and MDA.
Metrie	le Contr	rol		
	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. It was not re- ported if the worms were acclimated.
Metrie	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 an	l Analys	sis		
Metrie	21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section of the paper.
Metrie	22:	Reporting of Data	High	Exposure and control responses were reported in Figures 1-6. They were adequate for the outcomes of interest.
Metrie	23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability was reported in the figures.
Additional Comments: This p	ortion of	f the evaluation was on the effect of DBP of swere chosen as the outcomes of interest.	on SOD, CAT, F	POD, GSH, GST, and MDA in E. fetida. Mechanistic outcomes of biomarkers and
Avorall Auglity Do	torm	ination	Modium	

Study Citation:	Frances, S. I	Frances, S. P. (1994). Response of a chigger, eutrombicula-hirsti (acari, trombiculidae) to repellent and toxicant compounds in the laboratory. Journal of						
Duration: Exposure Route, Media, Path:	Medical Ent Overall Dura Terrestrial; N	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; Eutrombicula hirsti; Larvae						
Health Outcome:	Behavioral							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
HERO ID:	1341925	1341925						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported at Colbar in Melbourne, Australia. It was not re- ported if it was analytically verified.				
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 100%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the chiggers were allocated into study groups.				
Domain 2: Exposure Ch	araatarization							
Domain 5. Exposure Cr	Metric 7:	Experimental System/Test Media Preparation	Low	Minimal details were provided on the preparation of the test solutions. Serial dilutions were reported to be used, but little other information was provided.				
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered via 10 x 10cm cotton fabric. The test substance (0.3mL) was added and allowed to dry for 20h before 10-20 chigger larvae were placed in the test system. Chiggers were checked every three minutes until the mite could no longer move the length of its body. The duration was not reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in the study.				
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	The exposure duration was not reported. It was reported that the organisms were ob- served every three minutes, but it was unclear how long this continued for.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number and the spacing of the exposure groups was not reported. Only endpoint values were reported.				
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of an appropriate solvent control.				
Domain 4: Test Organis	m	— • • • • • •						
	Metric 13:	Test Organism Characteristics	High	Organisms were obtained from a culture colony originally obtained from larvae col- lected at Cowley Beach near Innisfail, northern Queensland, Australia.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.				
	Metric 15:	Conditions Number of Organisms and	Low	There were 10-20 chiggers tested at once. Tests were repeated three times at each con-				
		Replicates per Group	N	Contation.				
			Continued on next page					

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Dibutyl Phthalate

		col	ntinued from previous	page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Frances, S. F Medical Entr Overall Dura Terrestrial; N Invertebrate; Behavioral Dibutyl phth 1341925	P. (1994). Response of a chigger, eutrombicul omology 31(4):628-630. ation: 0 - 4 days (0-96h); Exposure Duration: J/A (e.g., injection); Not determined by study Arthropods; <i>Eutrombicula hirsti</i> ; Larvae alate (DBP)	a-hirsti (acari, trombic 0 - 4 days (0-96h) authors (i.e., chemical	ulidae) to repellent and toxicant compounds in the laboratory. Journal of of interest in exposure water, but unable to determine exact uptake route)
Domain		Metric	Rating	Comments
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	Medium	Feeding regimen was reported for the larvae, and they were kept at 27C in 75-80% rela-
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–behavior changes due to DBP exposure reported as ET50 and ET95 in terms of time to knock out.
	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 monitored for behavioral changes every three minutes until they no longer can move the length of their body.
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. It was not re- ported if any acclimation occurred.
	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	Probit analysis was reported to be used to determine ET50 and ET95 values.
	Metric 22:	Reporting of Data	Low	Only ET50 and ET95 values were reported in Table 2. Control response and any raw data were not 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 study w x 10cm cotto	as on the effect of DBP on repellent behavior on cloth. The study received an unacceptable r	in chigger E. hirsti. Or ank due to the lack of	ganisms were observed after being placed in the test chamber with the 10 nformation regarding exposure concentrations and duration.

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Toyo, Species, Age:	Frances, S. F Medical Ente Overall Dura Terrestrial; N	P. (1994). Response of a chigger, eutrombicutomology 31(4):628-630. ation: 0 - 4 days (0-96h); Exposure Duration: J/A (e.g., injection); Not determined by study	lla-hirsti (acari, trombicu 0 - 4 days (0-96h) / authors (i.e., chemical c	lidae) to repellent and toxicant compounds in the laboratory. Journal of of interest in exposure water, but unable to determine exact uptake route)			
Health Outcome	Behavioral	Arthropods, Eutromotcuta nirsti, Larvae					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1341925	1341925					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported at Colbar in Melbourne, Australia. It was not re- ported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 100%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the chiggers were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	Minimal details were provided on the preparation of the test solutions. Serial dilutions were reported to be used, but little other information was provided.			
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were administered via filter paper. The test substance (0.5mL) was added and allowed to dry before five chigger larvae were placed in the test system. Chiggers were monitored for 10 minutes for changes in behavior due to the repellant.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test substance was measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The exposure duration was reported to be 10 minutes, and this was adequate to see a response.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number and the spacing of the exposure groups was not reported. Only endpoint values were reported.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of an appropriate solvent control.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	High	Organisms were obtained from a culture colony originally obtained from larvae col- lected at Cowley Beach near Innisfail, northern Queensland, Australia.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were five chiggers tested at once. Tests were repeated at least seven times at each concentration.			
Domain 5: Outcome As	sessment						

Continued on next page ...

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1341925 Table: 2 of 2

		CO	ntinued from previous	page			
Study Citation:	Frances, S. I Medical Ent	P. (1994). Response of a chigger, eutrombicution omology 31(4):628-630.	la-hirsti (acari, trombicu	ilidae) to repellent and toxicant compounds in the laboratory. Journal of			
Duration:	Overall Dura	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; Arthropods; Eutrombicula hirsti; Larvae						
Health Outcome:	Behavioral						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1341925						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Feeding regimen was reported for the larvae, and they were kept at 27C in 75-80% relative humidity.			
	Metric 17:	Outcome Assessment Methodology	Uninformative	Repellency and mortality was combined to evaluate ED50 (effective dose) values - and the study did not specify what fraction died and what fraction were repelled.			
	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 monitored for behavioral changes for 10 minutes to determine if the repellant was effective.			
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. It was not re- ported if any acclimation occurred.			
	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	Probit analysis was reported to be used to determine ED50 values.			
	Metric 22:	Reporting of Data	Low	Only ED50 values were reported in Table 1. Control response and any raw data were not reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.			
Additional Comments:	This study was on the effect of DBP on repellent behavior in chigger E. hirsti. Organisms were observed after being placed in the test chamber treated filter paper. If the organisms remained in place, the repellent was successful. If they moved off the paper, the repellant did not work. The study received an unacceptable rank due to the lack of information regarding exposure concentrations and because the outcome evaluated was unclear (mortality combined with repellency).						

Overall Quality Determination

Uninformative

Study Citation: Duration:	Jensen, J., L fimetaria. Er Overall Dura	angevelde, van, J., Pritzl, G., Krogh, P. H. nvironmental Toxicology and Chemistry 20(ation: 4 - 10 days; Exposure Duration: 4 - 1	(2001). Effects (5):1085-1091. 0 days	of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia			
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: Chemical: HERO ID:	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile Mortality Dibutyl phthalate (DBP) 789786						
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 2: Exposure Ch	aractorization						
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	DBP 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 DBP at 0, 1, 5, 10, and 25 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			

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HERO ID: 789786 Table: 1 of 1

		contin	nued from previo	bus page	
Study Citation: Duration: Exposure Route, Media Dath:	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: 4 - 10 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				
Chemical: HERO ID:	Dibutyl phth 789786	alate (DBP)			
Domain		Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 juveniles per concentration with two replications.	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and/or environmental conditions and/or food, water, and 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 at the end of the one-week exposure.	
	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	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	vsis			
	Metric 21:	Statistical Methods	High	Details of statistical methods were reported in the methods and are appropriate for the study.	
	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. "Juvenile mortality was similar when exposed in multidisheswith 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 Quali	ty Deterr	nination	Medium		

Study Citation:	Jensen, J., L	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 Dura Terrestrial; S	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:	Invertebrates Mortality Dibutyl phth 789786	; Arthropods; <i>Folsomia fimetaria</i> ; Adult nalate (DBP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	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	Medium	Biological responses in the control were reported but there were limitations. "In some of the tests, mortality in the control samples exceeded 20%, which lessened the certainty of the test results".		
	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 degra- dation, but no effort was made to replenish the phthalate in soil. Concentrations of test substance were not measured during the study.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	DBP 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 DBP at 0, 100, 250, 500, 750, 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 Organisi	n					
	Metric 13:	Test Organism Characteristics	High	Male & female adult collembolans were used.		
	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	ge		

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Environmental Hazard Evaluation

HERO ID: 789786 Table: 1 of 2

		contin	ued from previ	ous page		
Study Citation:	Jensen, J., L fimetaria Fr	angevelde, van, J., Pritzl, G., Krogh, P. H.	(2001). Effects 5):1085-1091	of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia		
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	hemical of inter	est in exposure water, but unable to determine exact uptake route)		
Media, Path:	, .	,		I I I I I I I I I I I I I I I I I I I		
Taxa, Species, Age:	Invertebrate;	Arthropods; <i>Folsomia fimetaria</i> ; Adult				
Health Outcome:	Mortality	1 2 2 2				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	789786					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 male & 10 female per microcosm with four replicates per concentration.		
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and conditions were acceptable. 'Experimentswere conducted at a constant temperature (20oC), with a12:12 h light: dark regime. Animals were fed dried baker's yeast (15mg 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	vsis				
	Metric 21:	Statistical Methods	High	Details of statistical methods were reported in the methods and are appropriate for the study.		
	Metric 22:	Reporting of Data	High	The EC10/LC10/EC50/LC50 are reported for all endpoints.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Concentration reported. Co	on of DBP in soil was not measured during notrol mortality exceeded 20% (Fig 2).	or at the end of	the experiment. Environmental conditions (moisture content, pH, etc.) were not		
Avorall Auglit	v Dotorn	nination	Modium			

Study Citation: Duration: Exposure Route,	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Fol 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)						
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate Reproductiv Dibutyl phth 789786	nvertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult Reproductive/Teratogenic Dibutyl phthalate (DBP) 789786					
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	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	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 degra- dation, but no effort was made to replenish the phthalate in soil. Concentrations of test substance were not measured during the study.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	DBP 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 DBP at 0, 100, 250, 500, 750, 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						
2 511min 1. 105t 015dill5	Metric 13.	Test Organism Characteristics	High	Male & 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			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 10 male & 10 female per microcosm, with four replicates per concentration.			
Domain 5: Outcome As	sessment						

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Environmental Hazard Evaluation

HERO ID: 789786 Table: 2 of 2

		contin	nued from previo	us page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Jensen, J., L fimetaria. En Overall Dura Terrestrial; S Invertebrate; Reproductiv Dibutyl phth 789786	Angevelde, van, J., Pritzl, G., Krogh, P. H. nvironmental Toxicology and Chemistry 20(ation: 11 - 21 days; Exposure Duration: 11 Soil; Not determined by study authors (i.e., o ; Arthropods; <i>Folsomia fimetaria</i> ; Adult e/Teratogenic nalate (DBP)	(2001). Effects c (5):1085-1091. - 21 days chemical of intere	of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan Folsomia st in exposure water, but unable to determine exact uptake route)
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and conditions were 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	Medium	Reproduction was assessed by counting surviving juveniles at the end of 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	y / Variable Co	ntrol		
2 oniani or comoanany	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	tation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Details of statistical methods were reported in the methods and are appropriate for the study.
	Metric 22:	Reporting of Data	High	Adult reproduction data is given in Fig 2. The EC10/LC10/EC50/LC50 are reported for all endpoints (Table 1).
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			
Overall Quali	ty 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				
Duration: Exposure Route, Media. Path:	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: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 789786	Arthropods; <i>Folsomia fimetaria</i> ; Juvenile			
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	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				
Domain 5. Exposure en	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 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	DBP 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 DBP at 0, 1, 5, 10, and 25 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	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.	
	Metric 15:	Conditions Number of Organisms and	Low	20 organisms per exposure concentration, no replicates.	
		Replicates per Group			
		Contin	ued on next p	age	

HERO ID: 789786 Table: 1 of 2

		conun	ued from previo	ous page		
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					
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:	Invertebrate;	Arthropods; Folsomia fimetaria; Juvenile				
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789786					
Domain		Metric	Rating	Comments		
Domain 5: Outcome Ass	sessment					
Domain 5. Outcome As.	Metric 16:	Adequacy of Test Conditions	High	Organism housing & conditions acceptable. "Experiments were run at constant tempera- ture (200C), with a12:12 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	/ Variable Cor	ntrol				
Domain of Comounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	Weate 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 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	Low	EC10/LC10/EC50/LC50 are reported for all endpoints. But percent survival data was not given for each treatment group and control.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.		
Additional Comments:	None					

Overall Quality Determination

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. E	fimetaria. Environmental Toxicology and Chemistry 20(5):1085-1091.					
Duration:	Overall Dur	ation: 4 - 10 days; Exposure Duration: > 21	days				
Exposure Route,	Terrestrial;	Soil; Not determined by study authors (i.e., c	chemical of inte	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:	Developmen	ht/Growth					
Chemical:	Dibutyl phtl	nalate (DBP)					
HERO ID:	/89/86						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	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 5. Exposure Cr	Metric 7.	Experimental System/Test Media	Low	Experiments were conducted in small multidish chamber vessels with spiked soil. Be-			
	Wette 7.	Preparation	Low	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 test substance were not measured during the study.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Matria O.	Administration	T				
	Metric 9:	Concentration	Low	DBP 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 accentable (prenaration of spiked soil at begin-			
	incure ro.	Exposure Duration and Frequency	mgn	ning of experiment).			
	Metric 11:	Number of Exposure Groups/	High	"Test concentrations were DBP at 0, 1, 5, 10, and 25 mg/kg dry weight"			
		Spacing of Exposure Levels	C				
	Metric 12:	Testing at or Below Solubility Limit	N/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	Low	No reported acclimatization, but no evidence to suggest results impacted.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	20 organisms per exposure concentration, no replicates.			
Domain 5: Outcome As	sessment						

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		conti	nued 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	ation: 4 - 10 days; Exposure Duration: > 2	1 days			
Exposure Route,	Terrestrial; S	Soil; 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:	Invertebrate;	Invertebrate; Arthropods; Folsomia fimetaria; Juvenile				
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	789786					
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	High	Organism housing & conditions acceptable. "Experiments were run at constant tempera- ture (208C), with a 12:12 h light : dark regime."		
	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	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 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	EC10/LC10/EC50/LC50 are reported for all endpoints. DBP at the concentrations tested did not affect growth of juveniles (Fig 2).		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Medium			

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 (biot	transformation)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	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						
-	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	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 substance 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 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 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	Low	Minor uncertainties were identified regarding environmental conditions of the test sys- tem due to few details reported.		
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Dibutyl Phthalate

		contir	nued from previo	us page		
Study Citation:	Lenoir, A., 7 Science and	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 Dura	tion: 4 - 10 days; Exposure Duration: 4 - 10	0 days			
Exposure Route,	Terrestrial; N	J/A (e.g., injection); Dermal (topical applica	ation)			
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Invertebrate; Arthropods; Lasius niger; Adult				
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2347468					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcomes of interest with some uncertainty.		
	Metric 18:	Consistency of Outcome Assessment	Medium	There was incomplete reporting of minor details of outcome assessment protocol execu- tion.		
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 were no differences among groups, but few details were provided to confirm that.		
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 or limitations were identified in how the study characterized unexpected outcomes.		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Medium			

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Study Citation:	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita, PL of ONE 11(4):e0154675						
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; (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); Melo	idogyne incognite	a; Juvenile			
Health Outcome:	Behavioral	Behavioral					
HERO ID:	3350275	larate (DBP)					
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	Purity or 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 control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported other than to say control response was set at 100%.			
	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	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.			
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not 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 and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Three concentrations were used. For this experiment, a dose-response relationship was observed, though repellancy was not significantly different among any treatment. A wider range of concentrations should have been used.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
Domain 4. Tast Orregia							
Domain 4: Test Organis	Metric 13.	Test Organism Characteristics	High	The test organisms were adopted to described and were obtained from a reliable source			
	Metric 14	Acclimatization and Pretreatment	Low	The test organisms were accurately described and were obtained from a reliable source.			
	Metric 15:	Conditions Number of Organisms and	Medium	Three replicates were used.			
		Replicates per Group		r			
Domain 5: Outcome Ag	aaamont						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Conti	nued on next pa	ge			

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Environmental Hazard Evaluation

HERO ID: 3350275 Table: 1 of 2

		contin	ued from previ	ous page		
Study Citation:	Yang, G., Zh incognita, P	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita. PLoS ONE 11(4):e0154675				
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); Meloi	dogyne incognit	a; Juvenile		
Health Outcome:	Behavioral					
Chemical:	Dibutyl phth	nalate (DBP)				
HERO ID:	3350275					
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	Medium	Outcomes were assessed consistently across study groups, but few details were pro- vided.		
Domain 6: Confounding	r / 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	lysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were shown for each treatment but not the control group,		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This is for cl least a linear	hemotaxis. For this outcome, though only th dose-response relationship was observed. J	ree concentratio	ns were used and there were no statistically significant differences among them, at		

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Study Citation:	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incompite PLoS ONE 11(4):e0154675						
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; C	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); Mela	oidogyne inco	ognita; Juvenile			
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3350275						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce Matria 1:	Tast Substance Identity	Low	The shamical was identified by some only			
	Metric 1: Metric 2:	Test Substance Source	Low	The contract was identified by name only.			
	Metric 3:	Test Substance Purity	Low	Durity or grade of the test substance was not reported			
	mente 5.	Test Substance Furity	LUW	r unity of grade of the test substance was not reported.			
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	Low	The biological response of the negative control group was not reported other than to say control response was set at 100%.			
	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	Nothing in the study suggested that exposures were not 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 and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three concentrations were tested. A dose-response effect was not observed, and in fact mortality was highest in the middle concentration, and lowest in the lower and higher concentrations			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.			
		- ·	<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	Low	The study did not report whether test organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Three replicates were used.			
Domain 5: Outcome Ass	sessment	•					
Domain J. Outcome As	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.			
		Cart		t nore			
Continued on next page							

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HERO ID: 3350275 Table: 2 of 2

continued from previous page						
Study Citation:	Yang, G., Zh	ang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne				
	incognita. Pl	ncognita. PLoS ONE 11(4):e0154675.				
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); Melo	oidogyne inco	ognita; Juvenile		
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	3350275					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Medium	Outcomes were assessed consistently across study groups, but few details were pro-		
		Assessment		vided.		
Domain 6: Confounding	y / Variable Co	ntrol				
L. L	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 Durant	-4:					
Domain /: Data Present			TT' 1			
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were shown for each treatment but not the control group.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The nonlinearity in responses and lack of a dose-response relationship is not discussed.		
Additional Comments:	This form is	for mortality.				
Overall Qualit	ty Detern	nination	Low			

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Study Citation:	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne			
-	incognita. PLoS ONE 11(4):e0154675.			
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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); <i>Meloidogyne incognita</i> ; Embryo			
Health Outcome:	Development/Growth			
Chemical:	Dibutyl phthalate (DBP)			
HERO ID:	3350275			
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 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 other than to say control response was set at 100%.
	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	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not 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/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Three concentrations were used. A dose-response relationship was not observed. Hatch suppression was not significantly different in any treatment group. All three concentrations exhibited hatch suppression relative to control.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
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	Medium	Three replicates were used.
		Replicates per Group		
Domain 5: Outcome Ass	sessment		Ŧ	
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
Continued on next page				
Environmental Hazard Evaluation

HERO ID: 3350275 Table: 1 of 1

		conti	nued from p	revious page	
Study Citation:	Yang, G., Zh incognita. P	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne incognita. PLoS ONE 11(4):e0154675.			
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	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:	Invertebrate	Worms (e.g., Annelids, Nematodes); Mela	oidogyne inco	<i>ognita</i> ; Embryo	
Health Outcome:	Developmen	t/Growth	0.		
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	3350275				
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	Medium	Outcomes were assessed consistently across study groups, but few details were pro-	
		Assessment		vided.	
Demain (. Conformation					
Domain 6: Confounding	g / Variable Co	ntroi Confounding Variables in Test	Low		
	Metric 19:	Contounding variables in Test	Low	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	Low	Data for exposure-related findings were shown for each treatment but not the control group.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This form is	for the population abundance of the 8-day	test.		
Overall Qualit	ty Deterr	nination	Low		

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Study Citation:	dy Citation: Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne					
	incognita. Pl	LoS ONE 11(4):e0154675.	1 1			
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 2	I days	interest in supsours water but weakle to determine event untake route)		
Exposure Route, Modio Dothe	Terrestrial; S	refrestrar; son; Not determined by study autors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Tava Spacios Agas	Invariabrata	Worms (a.g. Annalida Namatadas); Mal	aidaanna ina	amita Invanila		
Taxa, Species, Age:	Davalonman	t/Growth	olaogyne incc	gnua, juvenne		
Chamical:	Dibutyl phth	alate (DBP)				
HFRO ID:	3350275					
Domain	5550215	Metric	Rating	Comments		
Domain 1: Test Substand	ce		Tuning			
	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 or grade of test substance 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	Low	The biological response of the negative control groups was not reported other than to say control response was set at 100%		
	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	Nothing in the study suggested that exposures were not 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/or exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Three concentrations were used. A dose-response relationship was not observed - hatch suppression was not significantly different in any treatment group. All three concentrations exhibited hatch suppression relative to control.		
	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	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	Three replicates were used		
		Replicates per Gloup				
Domain 5: Outcome Ass	sessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate		
		Cont	inued on nex	t page		

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Environmental Hazard Evaluation

HERO ID: 3350275 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Yang, G., Zh	Yang, G., Zhou, B., Zhang, X., Zhang, Z., Wu, Y., Zhang, Y., Lü, S., Zou, Q., Gao, Y., Teng, L. (2016). Effects of Tomato Root Exudates on Meloidogyne					
	incognita. Pl	incognita. PLoS ONE 11(4):e0154675.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
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:	Invertebrate;	Worms (e.g., Annelids, Nematodes); Meld	oidogyne inco	<i>ognita</i> ; Juvenile			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3350275						
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	Medium	outcomes were assessed consistently across study groups but few details were provided			
	Assessment						
Domain & Confounding	Variable Ca	ntanl					
Domain 6: Comounding	g / Variable Col	nuon Conformatina Variables in Test	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	vsis					
Domain 7: Data Frederic	Metric 21.	Statistical Methods	High	Statistical methods were adequately described			
	Metric 22.	Reporting of Data	Low	Data for exposure-related findings were shown for each treatment but not the control			
	Wether 22.	Reporting of Data	Low	group,			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
Additional Comments:	Population a	bundance of 8 day test.					
Overall Qualit	ty Deterr	nination	Low				

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues							
Duration: Exposure Route, Media. Path:	76(16):973-9 Overall Dura Terrestrial; F	76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Mortality Dibutyl phth 2219889	Invertebrate; Arthropods; <i>Spodoptera frugiperda</i> ; F1 generation; Larvae Mortality Dibutyl phthalate (DBP) 2219889						
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 DBP was not reported.				
	Metric 3:	Test Substance Purity	High	The 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.				
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the armyworms were allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	The test system used 150mL plastic cups with plastic caps. The DBP was dissolved into n-hexane at 1000mg/L. This was added to the food at 1mg/g. More information is needed on the test system.				
	Metric 8:	Consistency of Exposure Administration	Low	Plastic cups were used as the test chambers, so there is concern about the consistency of the exposure due to potential leaching and sorbing of the test substance.				
	Metric 9:	Measurement of Test Substance Concentration	Low	It is unclear if the DBP concentration in the food was measured at any point in the study. Analysis was done on the worms themselves.				
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration was reported to be until death in the adult phase. Authors also reported the use of the F1 moths to produce the F2 larvae. It is unclear how long the exposure actually was.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level, as the goal was not to observe a dose response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.				
Domain 4: Test Organis	m							
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The organisms were reported to be from an in-house colony at Laboratory of Pest Con- trol and Insect Maintenance at the University of Craxia do Sol.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 100 larvae placed individually into 150mL plastic cups.				

Dibutyl Phthalate

		col	ntinued from previous	page			
Study Citation:	Filho, D.N., fall armywor 76(16):973-9	Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues					
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: Not-	reported				
Exposure Route,	Terrestrial; F	Food/Diet; Dietary	1				
Media, Path:							
Taxa, Species, Age:	Invertebrate;	Arthropods; Spodoptera frugiperda; F1 gene	ration; Larvae				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2219889						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were kept at 25c with a relative humidity of 70% with a 14L:10D photope- riod. Organisms were fed, as the exposure was via diet, but the feeding regimen was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-mortality.			
	Metric 18:	Consistency of Outcome Assessment	Low	It was unclear when the organisms were assessed for mortalities.			
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 or other non-treatment-related factors across study groups. It was unclear 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							
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "Statistical Analysis" section of the paper.			
	Metric 22:	Reporting of Data	Low	Results were only described in the text.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.			
Additional Comments:	This portion of the evaluation was on the effect of DBP on armyworm mortality after exposure via diet. This is for the F1 generation. Mortality was selected as the outcome of interest. The study received an unacceptable ranking due to the lack of information on the study duration.						

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Citation: Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. ion: Overall Duration: Not-reported; Exposure Duration: Not-reported sure Route, a, Path: Terrestrial; Food/Diet; Dietary						
Taxa, Species, Age: Health Outcome:	Invertebrate; Arthropods; Spodoptera frugiperda; F1 generation; Larvae						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2219889						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be >99%.			
Domain 2: Test Design							
Domain 21 Teor Deorgi	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the armyworms were allocated into study groups.			
Domain 3. Exposure Ch	paracterization						
Domain 5. Exposure er	Metric 7:	Experimental System/Test Media Preparation	Low	The test system used 150mL plastic cups with plastic caps. The DBP was dissolved into n-hexane at 1000mg/L. This was added to the food at 1mg/g. More information is needed on the test system.			
	Metric 8:	Consistency of Exposure Administration	Low	Plastic cups were used as the test chambers, so there is concern about the consistency of the exposure due to potential leaching and sorbing of the test substance.			
	Metric 9:	Measurement of Test Substance	Low	It is unclear if the DBP concentration in the food was measured at any point in the study. Analysis was done on the worms themselves.			
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration was reported to be until death in the adult phase. Authors also reported the use of the F1 moths to produce the F2 larvae. It is unclear how long the exposure actually was.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level, as the goal was not to observe a dose response.			
		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 organisms were reported to be from an in-house colony at Laboratory of Pest Con- trol and Insect Maintenance at the University of Craxia do Sol.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 100 larvae placed individually into 150mL plastic cups.			
		Replicates per Group					

Domain 5: Outcome Assessment

Dibutyl Phthalate

		сог	ntinued from previou	s page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Filho, D.N., fall armywo 76(16):973-9 Overall Dura Terrestrial; F Invertebrate; ADME (biot Dibutyl phth 2219889	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F1 generation; Larvae ADME (biotransformation) Dibutyl phthalate (DBP) 2219889 			
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were kept at 25c with a relative humidity of 70% with a 14L:10D photope- riod. Organism were fed, as the exposure was via diet, but the feeding regimen was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–accumulation of DBP in armyworm tissue.	
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Male and female pupa were ground and an extraction was done. GC was used to analyzed DBP levels.	
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. It was unclear 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 Present	tation and Anal	ysis			
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "Statistical Analysis" section of the paper.	
	Metric 22:	Reporting of Data	High	Data for the exposure and control responses was presented in Figure 1 and was adequate for the outcome of interest.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was presented in figure 1.	
Additional Comments:	This portion selected as the	of the evaluation was on accumulation of D he outcome of interest. The study received an	BP in armyworm tissu unacceptable ranking	the after an exposure via diet. This is for the F1 generation. ADME was due to the lack of information on the study duration.	

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Filho, D.N., fall armywo 76(16):973- Overall Dur Terrestrial; I	Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of all armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 16(16):973-977. Dverall Duration: Not-reported; Exposure Duration: Not-reported Ferrestrial; Food/Diet; Dietary					
Taxa, Species, Age:	Invertebrate	; Arthropods; <i>Spodoptera frugiperda</i> ; F1 gen	eration; Larvae				
Chemical:	Dibutyl pht	Dibutyl phthalate (DBP)					
HERO ID:	2219889						
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 DBP was not reported.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be >99%.			
Domain 2: Test Design							
2 0111111 21 1000 2 001gi	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and was adequate for the outcome of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the armyworms were allocated into study groups.			
Domain 3: Exposure Ch	oractorization						
Domain 5. Exposure er	Metric 7:	Experimental System/Test Media Preparation	Low	The test system used 150mL plastic cups with plastic caps. The DBP was dissolved into n-hexane at 1000mg/L. This was added to the food at 1mg/g. More information is needed on the test system.			
	Metric 8:	Consistency of Exposure Administration	Low	Plastic cups were used as the test chambers, so there is concern about the consistency of the exposure due to potential leaching and sorbing of the test substance.			
	Metric 9:	Measurement of Test Substance	Low	It is unclear if the DBP concentration in the food was measured at any point in the study. Analysis was done on the worms themselves.			
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration was reported to be until death in the adult phase. Authors also reported the use of the F1 moths to produce the F2 larvae. It is unclear how long the exposure actually was.			
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level, as the goal was not to observe a dose response.			
		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 organisms were reported to be from an in-house colony at Laboratory of Pest Con- trol and Insect Maintenance at the University of Craxia do Sol.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 100 larvae placed individually into 150mL plastic cups.			
		Replicates per Group					

Domain 5: Outcome Assessment

Dibutyl Phthalate

		cor	ntinued from previou	s page			
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Filho, D.N., fall armywo 76(16):973-9 Overall Dura Terrestrial; F Invertebrate; Developmen Dibutyl phth 2219889	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F1 generation; Larvae Development/Growth Dibutyl phthalate (DBP) 2219889 					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were kept at 25c with a relative humidity of 70% with a 14L:10D photope- riod. Organism were fed, as the exposure was via diet, but the feeding regimen was not reported.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–weight, length, and width of the organisms.			
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Male and female pupae were measured and weighed, but details regarding this were limited.			
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. It was unclear 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 Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "Statistical Analysis" section of the paper.			
	Metric 22:	Reporting of Data	High	Data for the exposure and control responses was presented in Table 1 and was adequate for the outcome of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was presented in Table 1.			
Additional Comments:	This portion was selected	of the evaluation was on the effect of DBP or as the outcome of interest. The study received	his portion of the evaluation was on the effect of DBP on armyworm growth after exposure via diet. This is for the F1 generation. Development/growth ras selected as the outcome of interest. The study received an unacceptable ranking due to the lack of information on the study duration.				

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F2 generation; Larvae Development/Growth Dibutyl phthalate (DBP) 				
HERO ID:	2219889				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	Ce	Test Caletan and Identify	T		
	Metric 1:	Test Substance Source	Low	The test substance was identified by name only.	
	Metric 3:	Test Substance Purity	High	The purity was reported to be $>99\%$	
	incure 5.	Test Substance Funty	Ingn		
Domain 2: Test Design					
c	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.	
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 1 and was adequate for the outcome of interest.	
	Metric 6:	Randomized Allocation	Low	It was not reported how the armyworms were allocated into study groups.	
Domain 3: Exposure Cr	Metric 7:	Experimental System/Test Media Preparation	Low	The test system used 150mL plastic cups with plastic caps. The DBP was dissolved into n-hexane at 1000mg/L. This was added to the food at 1mg/g. More information is needed on the test system.	
	Metric 8:	Consistency of Exposure	Low	Plastic cups were used as the test chambers, so there is concern about the consistency of the avaguer due to potential lenghing and carbing of the test substance.	
	Metric 9:	Administration Measurement of Test Substance	Low	It is unclear if the DBP concentration in the food was measured at any point in the study. Analysis was done on the worms themselves	
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration was reported to be until death in the adult phase. Authors also reported the use of the F1 moths to produce the F2 larvae. It is unclear how long the exposure actually was.	
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level, as the goal was not to observe a dose response.	
	Metric 12:	Spacing of Exposure Levels 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 organisms were reported to be from an in-house colony at Laboratory of Pest Con-	
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 100 larvae placed individually into 150mL plastic cups.	
		Replicates per Group			

Domain 5: Outcome Assessment

Dibutyl Phthalate

		cor	ntinued from previou	s page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Filho, D.N., fall armywo 76(16):973-9 Overall Dura Terrestrial; F Invertebrate; Developmen Dibutyl phth 2219889	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F2 generation; Larvae Development/Growth Dibutyl phthalate (DBP) 2219889 			
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were kept at 25c with a relative humidity of 70% with a 14L:10D photope- riod. Organism were fed, as the exposure was via diet, but the feeding regimen was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–weight, length, and width of the organisms.	
	Metric 18:	Consistency of Outcome Assessment	Medium	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Male and female pupae were measured and weighed, but details regarding this were limited.	
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. It was unclear 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 Presen	tation and Anal	ysis			
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "Statistical Analysis" section of the paper.	
	Metric 22:	Reporting of Data	High	Data for the exposure and control responses was presented in Table 1 and was adequate for the outcome of interest.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was presented in Table 1.	
Additional Comments:	This portion was selected	of the evaluation was on the effect of DBP or as the outcome of interest. The study received	n armyworm growth a d an unacceptable ranl	fter exposure via diet. This is for the F2 generation. Development/growth sing due to the lack of information on the study duration.	

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F2 generation; Larvae ADME (biotransformation) 				
HERO ID:	2219889				
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 DBP was not reported.	
	Metric 3:	Test Substance Purity	High	The purity was reported to be >99%.	
Domain 2: Test Design					
Domain 21 Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.	
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- come of interest.	
	Metric 6:	Randomized Allocation	Low	It was not reported how the armyworms were allocated into study groups.	
Domain 3: Exposure Ch	aracterization				
Domain 5. Exposure Ci	Metric 7:	Experimental System/Test Media Preparation	Low	The test system used 150mL plastic cups with plastic caps. The DBP was dissolved into n-hexane at 1000mg/L. This was added to the food at 1mg/g. More information is needed on the test system.	
	Metric 8:	Consistency of Exposure Administration	Low	Plastic cups were used as the test chambers, so there is concern about the consistency of the exposure due to potential leaching and sorbing of the test substance.	
	Metric 9:	Measurement of Test Substance Concentration	Low	It is unclear if the DBP concentration in the food was measured at any point in the study. Analysis was done on the worms themselves.	
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration was reported to be until death in the adult phase. Authors also reported the use of the F1 moths to produce the F2 larvae. It is unclear how long the exposure actually was.	
	Metric 11:	Number of Exposure Groups/	N/A	There was only one exposure level, as the goal was not to observe a dose response.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 1. Test Organia	m				
Domain 4. 10st Organis	Metric 13:	Test Organism Characteristics	High	The organisms were reported to be from an in-house colony at Laboratory of Pest Con- trol and Insect Maintenance at the University of Craxia do Sol.	
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.	
	Metric 15:	Conditions Number of Organisms and	Medium	There were 100 larvae placed individually into 150mL plastic cups.	
		Replicates per Group			

Domain 5: Outcome Assessment

Dibutyl Phthalate

		col	ntinued from previou	s page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Filho, D.N., fall armywor 76(16):973-9 Overall Dura Terrestrial; F Invertebrate; ADME (biot Dibutyl phth 2219889	 Filho, D.N., I., Vieceli, N. C., Cardoso, E. M., Lovatel, E. R., Gonzatti, C. F., Marzotto, J. A., Montezano, D. G., Specht, A. (2013). Two generations of fall armyworm (Lepidoptera: Noctuidae) contamination by di-n-butylphthalate. Journal of Toxicology and Environmental Health, Part A: Current Issues 76(16):973-977. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Food/Diet; Dietary Invertebrate; Arthropods; <i>Spodoptera frugiperda</i>; F2 generation; Larvae ADME (biotransformation) Dibutyl phthalate (DBP) 2219889 				
Domain		Metric	Rating	Comments		
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were kept at 25c with a relative humidity of 70% with a 14L:10D photope- riod. Organism were fed, as the exposure was via diet, but the feeding regimen was not reported.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–accumulation of DBP in armyworm tissue.		
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Male and female pupa were ground and an extraction was done. GC was used to analyzed DBP levels.		
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. It was unclear 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 Presen	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "Statistical Analysis" section of the paper.		
	Metric 22:	Reporting of Data	High	Data for the exposure and control responses was presented in Figure 1 and was adequate for the outcome of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was presented in Figure 1.		
Additional Comments:	This portion selected as the test of the selected as the test of the selected as the selected	This portion of the evaluation was on accumulation of DBP in armyworm tissue after an exposure via diet. This is for the F2 generation. ADME was selected as the outcome of interest. The study received an unacceptable ranking due to the lack of information on the study duration.				

Overall Quality Determination

Uninformative

Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-					
Duration: Exposure Route, Media, Path:	penoids agai Overall Dura Terrestrial; A	enoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Perrestrial; Air; 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; Tyrophagus putrescentiae; Adult					
Health Outcome:	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323221						
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	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 negative control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported for the vapor phase tests.			
	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	A one-time dose was used, but few details were provided.			
	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	Eight concentrations were tested, but it is not clear if this was for all compounds.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via contact.			
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	There were 25-30 mites with 3-6 replicates.			
Domain 5: Outcome Ass	sessment						
		Conti	Continued on next page				

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HERO ID: 1323221 Table: 1 of 4

continued from previous page							
Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-					
	penoids agai	penoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., o	chemical of interes	t in exposure water, but unable to determine exact uptake route)			
Media, Path:						
Taxa, Species, Age:	Invertebrate;	Arthropods; <i>Tyrophagus putrescentiae</i> ; Ac	dult				
Health Outcome:	Mortality						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323221						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health, but actual measured condition values for control and exposed vessels were not reported.			
	Metric 17:	Outcome Assessment Methodology	High	Mites were considered dead if limbs did not move upon being prodded with a wooden dowel.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- 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	vsis					
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.			
	Metric 22:	Reporting of Data	Low	Only slope and LD50s were reported.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.			
Additional Comments:	table 3						
Overall Qualit	ty Detern	nination	Medium				

Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-					
	penoids agai	penoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Transferial N(A (a - injection)) Not determined by study such as (i.e., showing) of interest in successful with we had a determined successful and the sector of the					
Exposure Koute, Modia Dathy	Terrestrial; N	N/A (e.g., injection); Not determined by stuc	iy authors (i.e., c	nemical of interest in exposure water, but unable to determine exact uptake route)			
Tava Species Age	Invertebrate	Invertebrate: Arthropods: Tyrophagus nutrescentiae: Adult					
Health Outcome	Mortality	Mortality					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323221						
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	Purity or grade of the test substance were not reported.			
Domain 2: Test Design							
Domain 21 Teor Doorgi	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 for the vapor phase tests.			
	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	A one-time dose was used, but few details were provided.			
	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	Eight concentrations were tested, but it is not clear if this was for all compounds.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via contact.			
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	There were 25-30 mites with 3-6 replicates.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health, but actual measured condition values for control and exposed vessels were not reported.			
		Contin	nued on next pa	ge			

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HERO ID: 1323221 Table: 2 of 4

		contir	ued from previ	ous page		
Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h): Exposure Duration	n: 0 - 4 days (0-9	6h)		
Exposure Route,	Terrestrial; N	V/A (e.g., injection); Not determined by stud	ly authors (i.e., c	hemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	,					
Taxa, Species, Age:	Invertebrate;	Arthropods; Tyrophagus putrescentiae; Ad	ult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323221					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	Mites were considered dead if limbs did not move upon being prodded with a wooden dowel.		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
	Assessment			ited.		
Domain 6: Confoundin	a / Variabla Co	ntrol				
Domain 0. Comounding	g / Variable Col Metric 10:	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		
	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	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	Low	Only slope and LD50s were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	table 4					
Overall Quali	tv Deterr	nination	Medium			

Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-					
D (1	penoids agai	enoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557.					
Duration:	Overall Dura	Jverall Duration: 0 - 4 days (0-96n); Exposure Duration: 0 - 4 days (0-96n) Tomastrich N/A (a.g. inisistion). Not determined by study outbox, (i.g. shamical of interast in avragure water, but yrable to determine event writely route).					
Exposure Route, Modio Dothy	Terrestrial; F	N/A (e.g., injection); Not determined by stud	ly authors (i.e., c	nemical of interest in exposure water, but unable to determine exact uptake route)			
Tava Species Age	Invertebrate	Arthropods: Tyrophagus putrescentiae: Ad	ult				
Health Outcome	Mortality	Mortality					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1323221						
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	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 negative control group.			
	Metric 5:	Negative Control Response	High	In section 3.2 it is reported that "no mortality was observed in the ethanol-treated con-			
		c i	U	trols."			
	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			
	Metrie 7.	Prenaration	mgn	adequate detail.			
	Metric 8:	Consistency of Exposure	Medium	A one-time dose was used, but few details were provided.			
		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 and/or exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Eight concentrations were tested, but it is not clear if this was for all compounds.			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via contact.			
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.			
	Matria 15.	Conditions	Madiana				
	Metric 15:	Replicates per Group	Medium	There were 25-30 mites with 3-6 replicates.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels were not reported.			
		Contin	nued on next pa	ge			
			-				

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HERO ID: 1323221 Table: 3 of 4

		conti	nued from previo	us page		
Study Citation:	Tak, J. H., K	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: $0 - 4 \text{ days} (0-96)$	(h)		
Exposure Route,	Terrestrial; N	N/A (e.g., injection); Not determined by stud	dy authors (i.e., ch	memical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	,					
Taxa, Species, Age:	Invertebrate	; Arthropods; <i>Tyrophagus putrescentiae</i> ; Ad	lult			
Health Outcome:	Mortality					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1323221					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	High	Mites were considered dead if limbs did not move upon being prodded with a wooden dowel.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- 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	tation and Anal	lysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	Low	Only slope and LD50s were reported.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	table 2					
Overall Quali	ty Deterr	nination	Medium			

Study Citation:	Tak, J. H., K	ak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter-					
Duration:	overall Dura	2001ds against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-557. Everall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., c	hemical of intere	st in exposure water, but unable to determine exact uptake route)			
Media, Path:	.						
Taxa, Species, Age: Health Outcome:	Invertebrate;	Invertebrate; Arthropods; <i>Tyrophagus putrescentiae</i> ; Adult					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1323221						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		Ŧ				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2: Metric 3:	Test Substance Durity	Low	I he test substance identity was not analytically verified by the performing laboratory.			
	Metric 5.	Test Substance Funty	LOw	Furity 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 negative control group.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported for the vapor phase tests.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 3: Exposure Ch	aracterization		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	Medium	A one-time dose was used, but few details were provided.			
	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	Medium	Eight concentrations were tested, but it is not clear if this was for all compounds.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via contact.			
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.			
	M 15	Conditions					
	Metric 15:	Replicates per Group	Medium	There were 25-30 mites with 3-6 replicates.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health, but actual measured condition values for control and exposed vessels was not reported.			
Continued on next page							

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HERO ID: 1323221 Table: 4 of 4

Study Citation: Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic a penoids against Tyrophagus putrescentiae (Acari: Acaridae). Pest Management Science 62(6):551-5 Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Exposure Route, Media, Path: Tarax, Species, Age: Invertebrate; Arthropods; <i>Tyrophagus putrescentiae</i> ; Adult Health Outcome: Mortality Invertebrate; Arthropods; <i>Tyrophagus putrescentiae</i> ; Adult Health Outcome: Mortality Invertebrate; Arthropods; <i>Tyrophagus putrescentiae</i> ; Adult Health Outcome: Mortality Invertebrate; Arthropods; <i>Tyrophagus putrescentiae</i> ; Adult HERO ID: 1323221 Invertebrate; Consistency of Outcome Low Domain Metric 18: Consistency of Outcome Low Metric 18: Consistency of Outcome Low The study did not provide e conditions. Domain 6: Confounding / Variable Control Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Reporting of Data Low Only slope and LD50s were					
Duration: Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h) Exposure Route, Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but un Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Tyrophagus putrescentiae; Adult Health Outcome: Mortality Chemical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric 17: Outcome Assessment Methodology High Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Assessment Metric 19: Confounding Variables in Test Low Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Metric 22: Reporting of Data Low Only slope and LD50s were and Metric 22: Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes	Tak, J. H., Kim, H. K., Lee, S. H., Ahn, Y. J. (2006). Acaricidal activities of paeonol and benzoic acid from Paeonia suffruticosa root bark and monoter- penoids against Tyrophagus putrescentiae (Acari, Acaridae). Past Management Science 62(6):551,557				
Exposure Route, Media, Path: Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but un Media, Path: Taxa, Species, Age: Invertebrate; Arthropods; Tyrophagus putrescentiae; Adult Health Outcome: Mortality Chemical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Low The study did not provide e conditions. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Domain 7: Data Presentation and Analysis Kerporting of Data Low Only slope and LD50s were					
Media, Path: Invertebrate; Arthropods; Tyrophagus putrescentiae; Adult Taxa, Species, Age: Invertebrate; Arthropods; Tyrophagus putrescentiae; Adult Health Outcome: Mortality Chemical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric 17: Outcome Assessment Methodology High Metric 18: Consistency of Outcome Low Details regarding the execu ited. Assessment Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Metric 22: Reporting of Data Low Only slope and LD50s were ad Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes	nable to determine exact uptake route)				
Taxa, Species, Age: Invertebrate; Arthropods; Tyrophagus putrescentiae; Adult Health Outcome: Mortality Demical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric Metric Rating Metric 17: Outcome Assessment Methodology High Mites were considered dead dowel. Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low The study did not provide e conditions. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Reporting of Data Low Only slope and LD50s were ad Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes	I I I I I I I I I I I I I I I I I I I				
Health Outcome: Mortality Dhemical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low Design and Procedures conditions. Metric 20: Outcomes Unrelated to Exposure Medium Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Metric 22: Reporting of Data Low Metric 23: Explanation of Unexpected Outcomes High					
Chemical: Dibutyl phthalate (DBP) HERO ID: 1323221 Domain Metric Rating Metric 17: Outcome Assessment Methodology High Mites were considered dead dowel. Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Sessment Low The study did not provide e conditions. Domain 6: Confounding / Variable Control Confounding Variables in Test Low The study did not provide e conditions. Domain 7: Data Presentation and Analysis Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Metric 22: Reporting of Data Low Only slope and LD50s were ad Metric 23: Explanation of Unexpected Outcomes High Statistical methods were ad Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes					
HERO ID: 1323221 Domain Metric Rating Metric 17: Outcome Assessment Methodology High Mites were considered dead dowel. Metric 18: Consistency of Outcome Low Details regarding the execu ited. Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Low The study did not provide e conditions. Domain 7: Data Presentation and Analysis Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Metric 22: Domain 7: Data Presentation and Analysis Explanation of Unexpected Outcomes High Statistical methods were ad Metric 23: Explanation of Unexpected Outcomes High There were no unexpected Outcomes					
DomainMetricRatingMetric 17:Outcome Assessment MethodologyHighMites were considered dead dowel.Metric 18:Consistency of OutcomeLowDetails regarding the execu ited.Domain 6: Confounding / Variable ControlAssessmentited.Domain 6: Confounding / Variable ControlMetric 19:Confounding Variables in TestLowMetric 19:Confounding Variables in TestLowThe study did not provide e conditions.Metric 20:Outcomes Unrelated to ExposureMediumThere was no information iDomain 7: Data Presentation and AnalysisMetric 21:Statistical MethodsHighMetric 21:Statistical MethodsHighStatistical methods were ad Metric 23:Explanation of Unexpected OutcomesMetric 23:Explanation of Unexpected OutcomesHighThere were no unexpected outcomes					
Metric 17: Outcome Assessment Methodology High Mites were considered dead dowel. Metric 18: Consistency of Outcome Low Details regarding the executive. Domain 6: Confounding / Variable Control Ited. Ited. Metric 19: Confounding Variables in Test Low The study did not provide e conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Metric 23: Explanation of Unexpected Outcomes High There were no unexpected Outcomes	Comments				
Metric 18: Consistency of Outcome Assessment Low Details regarding the executive ited. Domain 6: Confounding / Variable Control Image: Confounding Variables in Test Low The study did not provide executive conditions. Metric 19: Confounding Variables in Test Low The study did not provide executive conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Domain 7: Data Presentation and Analysis Image: Configure of Data Low Only slope and LD50s were ad Metric 22: Metric 22: Reporting of Data Low Only slope and LD50s were High There were no unexpected outcomes	d if limbs did not move upon being prodded with a wooden				
Assessment ited. Domain 6: Confounding / Variable Control Ited. Metric 19: Confounding Variables in Test Low Design and Procedures conditions. Metric 20: Outcomes Unrelated to Exposure Medium Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods Metric 22: Reporting of Data Low Only slope and LD50s were and Metric 23: Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes	ation of the study protocol for outcome assessment were lim-				
Domain 6: Confounding / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Conditions. Low The study did not provide e conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the study did not provide e conditions. Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Metric 22: Reporting of Data Low Only slope and LD50s were Metric 23: Explanation of Unexpected Outcomes					
Metric 19: Confounding Variables in Test Low The study did not provide e conditions. Metric 19: Onfounding Variables in Test Low The study did not provide e conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Metric 22: Reporting of Data Low Only slope and LD50s were Metric 23: Explanation of Unexpected Outcomes High There were no unexpected on the statistical Metric 24:					
Design and Procedures conditions. Metric 20: Outcomes Unrelated to Exposure Medium There was no information i Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Reporting of Data Low Only slope and LD50s were Metric 23: Explanation of Unexpected Outcomes High There were no unexpected Outcomes	enough information to allow a comparison of environmental				
Metric 20: Outcomes Unrelated to Exposure Medium There was no information in the term of term of the term of the term of term of the term of term of term of the term of t					
Domain 7: Data Presentation and Analysis Metric 21: Statistical Methods High Statistical methods were ad Metric 22: Reporting of Data Low Only slope and LD50s were Metric 23: Explanation of Unexpected Outcomes High There were no unexpected outcomes	in the study to suggest differences among groups.				
Metric 21:Statistical MethodsHighStatistical methods were adMetric 22:Reporting of DataLowOnly slope and LD50s wereMetric 23:Explanation of Unexpected OutcomesHighThere were no unexpected outcomes					
Metric 22:Reporting of DataLowOnly slope and LD50s wereMetric 23:Explanation of Unexpected OutcomesHighThere were no unexpected outcomes	dequately described.				
Metric 23: Explanation of Unexpected Outcomes High There were no unexpected of	re reported.				
	outcomes.				
Additional Comments: table 3					
Overall Quality Determination Medium					

Study Citation: Duration: Exposure Route, Media, Path:	Lã,Kke, H., field experin Overall Dura Terrestrial; N	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Dermal (topical application)				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Achilla millefolium; Not Ap	pplicable (e.g., fu	ingi or algae studies) or Not Reported		
Health Outcome:	Skin & Com	nective Tissue				
Chemical:	Dibutyl phth	alate (DBP)				
	9430461					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	Metric 1:	Test Substance Identity	Low	Chemicals were identified as di-(2-ethyl hexyl)-phthalate (DEHP) and di-(n-butyl)- phthalate (DBP). No CASRN was provided.		
	Metric 2:	Test Substance Source	High	DBP was sourced from Fluka, Switzerland, and DEHP was sourced from Scandiflex, Ltd., Denmark. Analytical verification was conducted.		
	Metric 3:	Test Substance Purity	High	DPB purity was >99.5%. DEHP purity was >99%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Negative controls contained untreated and solvent-treated (acetone (5%) + water (95%) + Tween 20 (0.1%)).		
	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 test material was prepared consistently.		
	Metric 8:	Consistency of Exposure	Low	Spraying procedure was not well-defined.		
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test concentrations were measured in the leaves immediately following treatment (day 0) and 1, 3, 7, 15 days after treatment.		
	Metric 10:	Exposure Duration and Frequency	High	The one-time application followed by 15 days of monitoring seems adequate to deter- mine chemical uptake by plants.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was a single exposure concentration.		
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations ex- ceeded the water solubility limit in the original application. Application concentration was presented in terms of ug/cm^-2. A solvent was used, so this likely enhanced the sol- ubility of the test material.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The test species were thin-cuticle species (Sinapis alba L.), a thick cuticle species (Bras- sica napus), and a common, wild-growing species (Achillea millefolium L.). No source was provided for the seeds.		
Continued on next page						

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Dibutyl Phthalate

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path:	Lã,Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Dermal (topical application)				
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Achilla millefolium; Not Ap	plicable (e.g., fu	ngi or algae studies) or Not Reported	
Health Outcome:	Skin & Conn	lective Tissue			
Unemical:	0/30/81	alate (DBP)			
	9450401			~	
Domain	N	Metric	Rating	Comments	
	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	Twelve plants per species were utilized for the laboratory experiment. It was unclear whether there was a single replicate or multiple replicates with smaller numbers of plants.	
Domain 5: Outcome Ass	assmant				
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Organism housing, environmental conditions, food, water, and nutrients were conducive to maintenance of health and biomass loading was appropriate as shown in Table 1. Biomass loading was not described.	
	Metric 17:	Outcome Assessment Methodology	Low	Chlorotic spot assessment was not described in the methods section. However, this is an observational assessment.	
	Metric 18:	Consistency of Outcome Assessment	Low	Since methods were not described, it is uncertain whether assessment was consistent.	
Domain 6: Confounding	/ Variable Cor	atral			
Domain 0. Comounding	Metric 19	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions	
	filetile 19.	Design and Procedures	mgn	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 Data	tion on 1 Arr 1				
Domain /: Data Presenta	Metric 21.	ysis Statistical Methods	Low	Statistical analysis for chloronnull assessment was not adequately described in the meth	
		Staustical Michilous	LOW	ods.	
	Metric 22:	Reporting of Data	Medium	No data was shown for chlorotic spots. Results were described in the text on pages 8 and 13 of the pdf.	
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.	
Additional Comments:	This form applies to all three plant species and both chemicals. Note that results for Brassica treated with DEHP in this laboratory experiment were not found in the publication. The study reported the absorption of the test materials through leaf tissue following spray application to leaves. The authors conducted the test as a timecourse of a single dose (therefore no information about whether the exposure concentration affects the unterla). Evaporation				

conducted the test as a timecourse of a single dose (therefore no information about whether the exposure concentration affects the uptake). Evaporation from plants also characterized for some species and data from some species specified amount absorbed vs amount on surface of the plant. The authors tested the components of the test chamber (carbon air filter) as well as the plant.

Dibutyl Phthalate

		continued from previous page	
Study Citation:	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxic field experiments. Environmental Pollution Se	cological effects of Di-(2-ethyl hexyl)-ph pries A: Ecological and Biological 32(3):1	halate and Di-n-butyl-phthalate on higher plants in laboratory and 79-199.
Duration:	Overall Duration: 11 - 21 days; Exposure Dur	ation: 11 - 21 days	
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topi	cal application)	
Media, Path:			
Taxa, Species, Age:	Vegetation; Vascular Plants; Achilla millefoliu	m; Not Applicable (e.g., fungi or algae stu	udies) or Not Reported
Health Outcome:	Skin & Connective Tissue		
Chemical:	Dibutyl phthalate (DBP)		
HERO ID:	9430481		
Domain	Metric	Pating	Comments

Domain	Metric	Rating	Comments
Overall Quality Dete	rmination	Medium	

Page 780 of 983

Study Citation:	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and
	field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Media, Path:	
Taxa, Species, Age:	Vegetation; Vascular Plants; Achilla millefolium; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	9430481
D i	

Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	Low	Chemicals were identified as di-(2-ethyl hexyl)-phthalate (DEHP) and di-(n-butyl)- phthalate (DBP). No CASRN provided.
	Metric 2:	Test Substance Source	High	DBP was sourced from Fluka, Switzerland, and DEHP was sourced from Scandiflex, Ltd., Denmark. Analytical verification was conducted.
	Metric 3:	Test Substance Purity	High	DPB Purity >99.5%; DEHP Purity >99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls contained untreated and solvent-treated (acetone (5%) + water (95%) + Tween 20 (0.1%)).
	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 onium of Enpooure on	Metric 7:	Experimental System/Test Media	Medium	The test material was prepared consistently.
	Metric 8:	Preparation Consistency of Exposure	Low	Spraying procedure not well-defined.
	Metric 9:	Measurement of Test Substance	High	The test concentrations were measured in the leaves immediately following treatment (day 0) and 1, 3, 7, 15 days after treatment.
	Metric 10:	Exposure Duration and Frequency	High	One-time application followed by 15 day monitoring seems adequate to determine chemical uptake by plants.
	Metric 11:	Number of Exposure Groups/	N/A	Single exposure concentration.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit in original application. Application concentration was presented in terms of ug/cm^-2. A solvent was used so this likely enhanced the solubility of the test material.
Domain 4: Test Organisr	n			
	Metric 13:	Test Organism Characteristics	Low	The test species were thin-cuticle species (Sinapis alba L.), a thick cuticle species (Brassica napus), and a common, wild-growing species (Achillea millefolium L.). No source was provided for the seeds.
	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.
		Conti	nued on next pa	ge

Dibutyl Phthalate

		contin	ued from previ	ous page
Study Citation:	Lã,Kke, H., I	Rasmussen, L. (1983). Phytotoxicological e	effects of Di-(2-e	thyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; N	tion: 11 - 21 days; Exposure Duration: 11 - //A (e.g., injection); Dermal (topical applica	21 days tion)	logical 52(5):179-199.
Taxa, Species, Age: Health Outcome: Chemical:	Vegetation; V ADME (biot: Dibutyl phth	Vascular Plants; <i>Achilla millefolium</i> ; Not Ap ransformation) alate (DBP)	plicable (e.g., fu	ngi or algae studies) or Not Reported
HERO ID:	9430481		D. (*	
Domain	Metric 15:	Number of Organisms and Replicates per Group	Low	12 plants per species utilized for the laboratory experiment per species; unclear whether this was a single replicate or multiple replicate with smaller numbers of plants.
Domain 5: Outcome Ass	Metric 16:	Adequacy of Test Conditions	Medium	Organism housing, environmental conditions, food, water, and nutrients were conducive to maintenance of health and biomass loading was appropriate as shown in Table 1. Biomass loading not described.
	Metric 17:	Outcome Assessment Methodology	High	Sampling, extraction, and measurement of chemicals from plant material and from car- bon air filters in the chamber described adequately. The units expressed by the authors are in ug chemical per plant. The authors did not characterize metabolic products or translocation of the material throughout the plant.
	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 Cor	itrol		
Domain of 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 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 Analy	ysis		
	Metric 21:	Statistical Methods	Low	Authors performed a time course but did not report significant differences in chemical concentration per plant. Approximate data points provided in Figures 1-5.
	Metric 22:	Reporting of Data	Medium	Data for treated plants as ug chemical per plant shown in Figures 1-5 but no data for controls was represented.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.
Additional Comments:	This form ap	plies to all three plant species and both test	t chemicals. Not	e that results for Brassica treated with DEHP in this laboratory experiment were

ditional Comments: This form applies to all three plant species and both test chemicals. Note that results for Brassica treated with DEHP in this laboratory experiment were not found in the publication. Study reported the absorption of the test materials through leaf tissue following spray application to leaves. The authors conducted the test as a timecourse of a single dose (therefore no information about whether the exposure concentration affects the uptake). Evaporation from plants also characterized for some species and data from some species specified amount absorbed vs amount on surface of the plant. The authors tested the components of the test chamber (carbon air filter) as well as the plant.

Overall Quality Determination

Medium

Dibutyl Phthalate

		continued from previous page					
Study Citation:	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxi field experiments. Environmental Pollution Se	cological effects of Di-(2-ethyl hexyl)-ph eries A: Ecological and Biological 32(3):1	thalate and Di-n-butyl-phthalate on higher plants in laboratory and 179-199.				
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topical application)						
Media, Path:							
Taxa, Species, Age:	Vegetation; Vascular Plants; Achilla millefoliu	m; Not Applicable (e.g., fungi or algae st	udies) or Not Reported				
Health Outcome:	ADME (biotransformation)						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	9430481						
Domain	Metric	Rating	Comments				

Study Citation:	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and
	field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Media, Path:	
Taxa, Species, Age:	Vegetation; Vascular Plants; Achilla millefolium; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Photosynthesis
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	9430481

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	Chemicals were identified as di-(2-ethyl hexyl)-phthalate (DEHP) and di-(n-butyl)- phthalate (DBP). No CASRN provided.
	Metric 2:	Test Substance Source	High	DBP was sourced from Fluka, Switzerland, and DEHP was sourced from Scandiflex, Ltd., Denmark. Analytical verification was conducted.
	Metric 3:	Test Substance Purity	High	DPB Purity >99.5%; DEHP Purity >99%.
Domain 2: Test Design				
2 oniun 21 1000 2 oorgi	Metric 4:	Negative Controls	High	Negative controls contained untreated and solvent-treated (acetone (5%) + water (95%) + Tween 20 (0.1%)).
	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 011111 01 2.1905010 01	Metric 7:	Experimental System/Test Media	Medium	The test material was prepared consistently.
	Metric 8:	Consistency of Exposure	Low	Spraying procedure not well-defined.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	The test concentrations were measured in the leaves immediately following treatment (day 0) and 1, 3, 7, 15 days after treatment.
	Metric 10:	Exposure Duration and Frequency	High	One-time application followed by 15 day monitoring seems adequate to determine chemical uptake by plants.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Single exposure concentration.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit in original application. Application concentration was presented in terms of ug/cm^-2. A solvent was used so this likely enhanced the solubility of the test material.
Domain 4. Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The test species were thin-cuticle species (Sinapis alba L.), a thick cuticle species (Brassica napus), and a common, wild-growing species (Achillea millefolium L.). No source was provided for the seeds.
	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.
		Conti	nued on next pa	

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 9430481 Table: 3 of 3

		contin	ued from previ	ous page			
Study Citation: Duration: Exposure Route, Media, Path:	Lã,Kke, H., field experin Overall Dur; Terrestrial; N	Lä,Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; N/A (e.g., injection); Dermal (topical application)					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Achilla millefolium; Not Ap	plicable (e.g., fu	ingi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	-Photosynthesis					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	9430481						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	12 plants per species utilized for the laboratory experiment per species; unclear whether			
		Replicates per Group		this was a single replicate or multiple replicate with smaller numbers of plants.			
Domain 5: Outcome As	sessment						
Domain 5. Outcome ras	Metric 16:	Adequacy of Test Conditions	Medium	Organism housing, environmental conditions, food, water, and nutrients were conducive to maintenance of health and biomass loading was appropriate as shown in Table 1. Biomass loading not described.			
	Metric 17:	Outcome Assessment Methodology	High	Chlorophyll analysis was described in the Analytical techniques section.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.			
		4.1					
Domain 6: Confounding	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	Metric 21:	Statistical Methods	Low	Statistical analysis for chlorophyll assessment was not adequately described in the meth- ods.			
	Metric 22:	Reporting of Data	Low	No data was shown for chlorophyll analysis. Brief results were described in the text on page 13 of the pdf.			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.			
Additional Comments:	This form ag found in the the test as a also charact components	pplies to all three plant species and both che publication. Study reported the absorption of timecourse of a single dose (therefore no int erized for some species and data from some of the test chamber (carbon air filter) as well	emicals. Note th f the test materia formation about e species specif l as the plant.	hat results for Brassica treated with DEHP in this laboratory experiment were not ils through leaf tissue following spray application to leaves. The authors conducted whether the exposure concentration affects the uptake). Evaporation from plants ied amount absorbed vs amount on surface of the plant. The authors tested the			
Overall Quali	ty Deterr	nination	Medium				

Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth						
Duration: Exposure Route, Media Path:	Overall Dura Terrestrial; V	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) 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:	Vegetation; V	Vascular Plants; Avena sativa; cv Victory;	Not Applicab	le (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth	11				
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5551990						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	Metric 1:	Test Substance Identity	High	Chemical name and structure were used to identify the test chemical.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.			
Domain 2: Test Design							
c	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.			
	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 test concentrations were not measured. Vessel material was not			
			Ŧ	reported.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	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 suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations were used, but a wide range was tested.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Exposure was via an aqueous test substance solution, but the carrier and quantities of material were not reported.			
Domain 4: Test Organist	n						
rest organisi	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	exposed groups. There were fifteen coleoptile sections per treatment, but the number of 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.			
		Cont	inued on nex	at page			

Dibutyl Phthalate

		conti	nued from p	previous page			
Study Citation:	Isogai, Y., K regulators. S	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.					
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	on: 0 - 4 days	(0-96h)			
Exposure Route,	Terrestrial; V	Vater; Not determined by study authors (i.e	e., chemical o	of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Avena sativa; cv Victory; 1	Not Applicab	ble (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5551990						
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	Low	Details regarding the execution of the study protocol for outcome assessment were lim- 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	vsis					
	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	High	Results were reported in Tables 5 and 6 for the control response and the exposure re- sponses. The data was appropriate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion of interest.	of the study was on the plant growth promo	oting activity	of DBP on Avena sativa-cv Victory. Development/Growth was selected as the outcome			
Overall Quali	ty Detern	nination	Low				

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:	Frontiers of Overall Dura	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	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; Avena sativa; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Unemical:	Dibutyl phth	alate (DBP)					
Domain	2913800	Matria	Dating	Comments			
Domain 1: Test Substan	ce	Metric	Katilig	Comments			
Domain 1. Test Substan	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	N . · ·		TT' 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 now 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		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		response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds were exposed via soil.			
Domain 4: Test Organis	m						
5	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-			
	metho io.	Replicates per Group	mearan	ize toxicological effects.			
		· · · ·					
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.			
Continued on next page							

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 2915866 Table: 1 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 & Env					
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:	Dibutyl phth	nalate (DBP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment	U	, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
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 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 were adequate to determine values for the endpoint.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	Pigment con	itent"The results indicate that longer period	ls of cultivati	on of the test plants may make it easier to interpret the changes in pigment contents."		
Overall Quali	ty Deterr	nination	High			

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	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>Avena sativa</i> ; Embryo Reproductive/Teratogenic							
Chemical: HERO ID:	Dibutyl phthalate (DBP) 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								
2 onian 21 Test 2 osign	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:	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							
	Metric 13:	Test Organism Characteristics	High	The test seeds were adequately described and were obtained from a reliable source.				
	Metric 14:	Acclimatization and Pretreatment	Hıgh	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 A -								
Domain 5. Outcome As	Metric 16.	A dequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism				
	wieute to:	Aucquacy of rest Conditions	nigii	health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	Hıgh	Outcomes were assessed consistently across study groups.				

Dibutyl Phthalate

continued from 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.						
D (1	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; 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:	Reproductive/Teratogenic						
Chemical:	Dibutyl phthalate (DBP)						
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	High	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:	This form represents the germination rate results presented in Table 1 for Avena sativa with DBP exposure.						

Overall Quality Determination

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>Avena sativa</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 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	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						
2 Sinum 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		
	whether 11.	Spacing of Exposure Levels	mgn	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 Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to character- ize toxicological effects.		
Domain 5: Outcome Ass	Domain 5: Outcome Assessment					
	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.		
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Dibutyl Phthalate

continued from previous page						
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.				
	Frontiers of	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; Avena sativa; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
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				
	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	High	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 Qualit	y Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Dueck, T. A.	., Dijk, Van, C. J., David, F., Scholz, N., Y	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant			
	species. Che	species. Chemosphere 53(8):911-920.					
Duration: Exposure Route	Terrestrial: Air: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact untake route)						
Media, Path:	referrial, All, Not determined by study autions (i.e., enermear of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:	Vegetation; V	Vegetation; Vascular Plants; Brassica campestris; chinensis; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1302103						
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	LOW	Purity or grade of test substance were not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.			
	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		TT' 1				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum.			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups based on timed samplings			
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."			
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-			
		Spacing of Exposure Levels	.	tration gradient is reported as both actual and nominal within table 1.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.			
Domain 1: Test Organic	m						
Domain 4: Test Ofganis	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.			
		Cont	inued on nev	xt nage			
		Cont		st page			

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 2

		conti	nued from p	previous page		
Study Citation:	Dueck, T. A	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.				
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	davs			
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., o	chemical of i	interest in exposure water, but unable to determine exact uptake route)		
Media. Path:	,	,				
Taxa, Species, Age:	Vegetation:	Vascular Plants: Brassica campestris: chine	ensis: Not Ar	pplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	1302103					
Domain		Metric	Rating	Comments		
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Four plants per treatment and no replicates reported, "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP.		
Domain 5: Outcome As	sessment					
Domain 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.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Outcomes were assessed consistently across study groups but few details were reported.		
Domain 6: Confounding	y / Variable Cou	ntrol				
Domain of Comountaing	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	veis				
Domain 7. Data i resent	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:	This form re	presents growth outcomes associated with	Dry Weight	reported for Brassica shoot and roots within Figure 3 on page 6/10.		
	Deter		TT: -1			
Overall Quality	ly Detern	nination	High			

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Study Citation:	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant						
Duration	species. Che	species. Chemosphere 53(8):911-920.					
Duration: Exposure Route	Terrestrial: A	1001: > 21 days; Exposure Duration: > 21	t days chemical of i	nterest in exposure water, but unable to determine exact untake route)			
Media, Path:	Terresultar, A	An, Not determined by study autions (i.e.,		increst in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation: V	Vascular Plants: <i>Brassica campestris</i> : chine	ensis: Not An	nlicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biot	ransformation)	, 1 (ot 1 1p	provide (e.g., rung, or algae statics) of reported			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1302103						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	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	Purity or 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, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.			
	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 2: Exposure Ch	araatarization						
Domain 5. Exposure Cha	Matric 7:	Experimental System/Test Media	High	The experimental system and methods for propagation of test media wave described in			
	Mettic 7.	Propagation	mgn	adequate detail. The chambers were constructed of "hardened glass" and aluminum			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups based on timed sam-			
		Administration	ingn	plings.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."			
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-			
	Metric 12.	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/Δ	tration gradient is reported as both actual and nominal within table 1.			
	Mettre 12.	Testing at of Below Solubility Emili	14/11				
Domain 4: Test Organisr	n						
	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but the source was not explicitly reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.			
		Cont	inued on nex	t page			

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Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

		conti	nued from p	previous page		
Study Citation:	Dueck, T. A	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Air; 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; Brassica campestris; chine	ensis; Not Ap	pplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	1302103					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were four plants per treatment and no replicates reported. "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP."		
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 Assessment	Medium	Outcomes were assessed consistently across study groups but few details were reported.		
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	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 in Table 2.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	This form is	for ADME (biotransformation) assessment	t of DBP cor	ncentration in leaf tissue.		
Overall Qualit	ty Detern	nination	High			

Study Citation:	Kong, X., Jii	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable					
Duration: Exposure Route, Madia Path:	ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake						
Taxa. Species. Age:	Vegetation: V	Vegetation; Vascular Plants; Brassica napus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Photosynthesis-Nutritional and Metabolic					
Chemical:	Dibutyl phth	alate (DBP)	0				
HERO ID:	4829418	4829418					
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	High	The test substance was identified by HPLC.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was 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 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 concentration. Exposure was conducted in a greenhouse with seedlings contained in ceramic pots.			
	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 Concentration	Low	Supplemental data needed to assess this metric was unavailable, therefore this metric score reflects the details provided in the study being reviewed			
	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	High	The number of exposure groups and spacing of exposure levels was suitable			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil			
Domain 4: Test Organis							
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Medium	Source was suitable, age of seedlings was not reported			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and			
		Conditions		exposed groups			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 seedlings with six replicates was reasonable			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate			
		Contin	nued on next pa				

Environmental Hazard Evaluation

HERO ID: 4829418 Table: 1 of 3

		contin	ued from previ	ous page		
Study Citation:	Kong, X., Ji ecosystem. J	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 d	days			
Exposure Route,	Terrestrial; S	Soil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation; Vascular Plants; Brassica napus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Cell signa	aling/function-P	hotosynthesis-Nutritional and Metabolic		
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	4829418					
Domain		Metric	Rating	Comments		
	Metric 17:	Outcome Assessment Methodology	Low	Few details regarding the execution of the study protocol for outcome assessment were 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 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		
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:	None					

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 4829418 Table: 2 of 3

Study Citation:	Kong, X., Jir	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable						
Duration: Exposure Route, Media. Path:	ecosystem. J Overall Dura Terrestrial; S	ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake Vegetation; Vascular Plants; <i>Brassica napus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age:	Vegetation; V							
Health Outcome:	ADME (biot	ransformation)						
Chemical: HFRO ID:	Dibutyl phth							
Domain	4029410	Matria	Dating	Comments				
Domain 1: Test Substand	ce.	Methe	Katilig	Comments				
Domain 1. Test Substant	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only				
	Metric 2:	Test Substance Source	High	The test substance was identified by HPLC.				
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was 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 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 concentration. Exposure was conducted in a greenhouse with seedlings contained in ceramic pots.				
	Metric 8:	Consistency of Exposure	Low	Only general methods of exposure administration were reported so assessment was difficult to determine				
	Metric 9:	Measurement of Test Substance Concentration	Low	Supplemental data needed to assess this metric was unavailable, therefore this metric score reflects the details provided in the study being reviewed				
	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 was suitable				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via soil				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	Source was suitable, age of seedlings was not reported				
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	exposed groups 10 seedlings with six replicates was reasonable				
Domain 5: Outcome Ass	sessment							
2 sinuin 51 Guteonie 715	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate				
	Metric 17:	Outcome Assessment Methodology	Low	Few details regarding the execution of the study protocol for outcome assessment were reported.				
	Continued on next page							

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HERO ID: 4829418 Table: 2 of 3

		contin	ued from previ	bus page		
Study Citation:	Kong, X., Ji ecosystem.	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150.				
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: > 21 d	days			
Exposure Route,	Terrestrial; S	Soil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica napus; Not Applica	able (e.g., fungi	or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	4829418					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-		
		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		
	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	lysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described		
	Metric 22:	Reporting of Data	Low	Supplemental data needed to assess this metric was unavailable, therefore this metric score reflects the details provided in the study being reviewed		
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained		
Additional Comments:	None					
Overall Quali	ty Deterr	nination	Medium			

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HERO ID: 4829418 Table: 3 of 3

	Wether 12.	Testing at of Below Solubility Linit	INA				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via soil.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels was suitable.			
	Metric 10.	Concentration Exposure Duration and Frequency	Luw	suppremental data nececi to assess this metric was unavariable, therefore this metric score reflects the details provided in the study being reviewed.			
	Metric 8:	Consistency of Exposure Administration Measurement of Test Substance	Low	Only general methods of exposure administration were reported so assessment was difficult to determine.			
Domain 3: Exposure Cl	naracterization Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The exposure was conducted in a greenhouse with seedlings contained in ceramic pots.			
	Metric 6:	Kandomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
Domain 2: Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.			
	Metric 2:	Test Substance Source	High	The test substance was identified by HPLC.			
Domain 1: Test Substar	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
Domain		Metric	Rating	Comments			
Chemical: HERO ID:	Dibutyl phth 4829418	Dibutyl phthalate (DBP) 4829418					
Media, Path: Taxa, Species, Age: Health Outcome:	Vegetation; Vascular Plants; <i>Brassica napus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth						
Duration: Exposure Route,	ecosystem. J Overall Dura Terrestrial; S	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake					
Study Citation: Duration: Exposure Route, Media. Path:	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegeta ecosystem. Journal of Hazardous Materials 353(Elsevier):142-150. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake						

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HERO ID: 4829418 Table: 3 of 3

		contin	ued from previ	ous page		
Study Citation:	Kong, X., Ji ecosystem.	Kong, X., Jin, D., Jin, S., Wang, Z., Yin, H., Xu, M., Deng, Y. (2018). Responses of bacterial community to dibutyl phthalate pollution in a soil-vegetable cosystem. Journal of Hazardous Materials 353(Elsevier):142-150.				
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Terrestrial; S	Soil; Root uptake				
Media, Path:		-				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica napus; Not Applic	able (e.g., fungi	or algae studies) or Not Reported		
Health Outcome:	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	4829418					
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- 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	tation and Anal	lysis				
	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:	None					
Overall Quali	ty Deterr	nination	Medium			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Hardwick, R	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.							
Duration:	Overall Dura	werall Duration: > 21 days; Exposure Duration: > 21 days errestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Exposure Route, Media, Path:	Terrestrial; A	Air; Not determined by study authors (i.e., cho	emical of interest in expo	osure water, but unable to determine exact uptake route)					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V Mortality Dibutyl phth 5678863	Vegetation; Vascular Plants; <i>Brassica oleracea</i> ; Derby Day cultivar; Juvenile Mortality Dibutyl phthalate (DBP) 5678863							
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	Metric 1:	Test Substance Identity	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP is the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.					
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.					
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported. The					
Domain 2: Test Design									
	Metric 4:	Negative Controls	High	It was reported there was negative control that contained zero plastic.					
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported. Several different plas- tics were reported to be testing, many of which had ND or a trace of DBP, but these did not appear to be the true negative control response.					
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.					
	, . <i>.</i> .								
Domain 3: Exposure Ch	aracterization	E-mail - Madia	T						
	Metric 7.	Preparation	Löw	ameter dwarf pots with 4 plants each. The plastic was suspended on a wire at the top of the cuvette that was ventilated. There was a non-plastic control for each type of plastic used. Measurements were taken of the DBP in the air. It is uncertain if the exposure was to DBP alone since other chemicals are involved in making plastic.					
	Metric 8:	Consistency of Exposure Administration	Medium	The exposure was reported to be for 4 weeks for all plants. All the plants were visually assessed for 1 month post sowing. All seedling were raised in 115mm diameter dwarf pots with 4 plants per pot.					
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.					
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 4 weeks. This appeared adequate to observe the outcome of interest.					
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, to compare a glazing using DBP to a glazing using DIDP and to other plastics potentially plasticized with DBP.					
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solu- bility limit.					

Domain 4: Test Organism

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 5678863 Table: 1 of 6

		CO	ntinued from previous	page				
Study Citation:	Hardwick, R emitted from	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.						
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21 da	ays					
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., che	emical of interest in expo	osure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica oleracea; Derby Da	y cultivar; Juvenile					
Health Outcome:	Mortality							
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5678863							
Domain		Metric	Rating	Comments				
	Metric 13:	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.				
	Metric 15:	Conditions Number of Organisms and	Low	For each plastic tested, there were three replicates. In each cuvette, there were 4 plants				
		Replicates per Group		per pot.				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	Low	The plants were kept at 20C during the day and 15C at night, but little other details were provided on environmental conditions.				
	Metric 17:	Outcome Assessment Methodology	Low	It was reported the plants were visually assessed for signs of toxicity 4 weeks after sow- ing, but details regarding the assessment were not provided.				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. Plants were assessed for signs of toxicity, but this protocol was not described.				
Domain 6: Confounding	y / Variable Co	ntrol						
Domain 0. Comountaing	Matria 10:	Confounding Variables in Test	Low	The study did not mayide 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 Present	ation and Anal	veie						
Domain 7. Data Meselli	Matric 21.	Statistical Mathada	Uninformativa	Statistical analysis for this partian of the study was not conducted. Desults were not				
	Metric 21:	Statistical Methods	Uninformative	statistical analysis for this portion of the study was not conducted. Results were not presented in a manner that allowed for statistical analysis.				
	Metric 22:	Reporting of Data	Medium	Results for the exposures were provided in Table 2, but negative control results were not reported.				
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.				
Additional Comments:	Metric 23: This portion cuvette. Seve	Explanation of Unexpected Outcomes of the evaluation was conducted in glass cuve eral plastics were tested. It was reported that a	Low ettes. The test plastic was control was run for each	Insufficient information was provided to determine if excessive outcomes occurred. s suspended from the top of the cuvette and chemicals vapor type of plastic, but control results were not reported. This st				

an unacceptable ranking due to the lack of statistical analysis. There is also concern that other chemicals in the plastic could be vaporizing and causing toxicity as well. The study authors only tested for phthalates. Authors reported visually assessing the plants 1 month after sowing. Details regarding this protocol were not provided. The outcomes assessed for were mortality, growth, and chlorosis.

Overall Quality Determination

Dibutyl Phthalate

Uninformative

Study Citation:	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate			
Duration	emitted from Overall Dura	certain plastics. Annals of Applied Biolocition: > 21 days: Exposure Duration: > 2	ogy 105(1):97-105. 1 days	
Exposure Route.	Terrestrial: A	ir: Not determined by study authors (i.e.,	chemical of interest in exposu	e water, but unable to determine exact uptake route)
Media, Path:	,		······	· · · · · · · · · · · · · · · · · · ·
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Brassica oleracea; Derby	Day cultivar; Juvenile	
Health Outcome:	Development	/Growth		
Chemical:	Dibutyl phtha	alate (DBP)		
HERO ID:	5678863			
Domain		Metric	Rating	Comments
Domain 1: Test Substance			т	
	Metric 1:	Test Substance Identify	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP is the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.
	Metric 3:	Test Substance Purity	LOW	The purity of the DBP was not reported. The
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported there was negative control that contained zero plastic.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported. Several different plas- tics were reported to be testing, many of which had ND or a trace of DBP, but these did not appear to be the true negative control response.
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.
Domain 3: Exposure Ch	aracterization			
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media Preparation	Low	The test system was reported to be conducted in glass cuvettes containing 115mm di- ameter dwarf pots with 4 plants each. The plastic was suspended on a wire at the top of the cuvette that was ventilated. There was a non-plastic control for each type of plastic used. Measurements were taken of the DBP in the air. It is uncertain if the exposure was to DBP alone since other chemicals are involved in making plastic.
	Metric 8:	Consistency of Exposure Administration	Medium	The exposure was reported to be for 4 weeks for all plants. All the plants were visually assessed for 1 month post sowing. All seedling were raised in 115mm diameter dwarf pots with 4 plants per pot.
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 4 weeks. This appeared adequate to observe the outcome of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, to compare a glazing using DBP to a glazing using DIDP and to other plastics potentially plasticized with DBP.
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solubility limit.
Domain 4: Test Organisr	n			
	Metric 13:	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported.
			Continued on next page	

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Environmental Hazard Evaluation

HERO ID: 5678863 Table: 2 of 6

R. C., Cole, R. A., Fyfield, T. P. (1984). Injur m certain plastics. Annals of Applied Biology ration: > 21 days; Exposure Duration: > 21 da Air; Not determined by study authors (i.e., che ; Vascular Plants; <i>Brassica oleracea</i> ; Derby Da ent/Growth thalate (DBP) <u>Metric</u> <u>Acclimatization and Pretreatment Conditions</u> Number of Organisms and <u>Replicates per Group</u>	ry to and death of cabba 105(1):97-105. ays emical of interest in expo y cultivar; Juvenile Rating Low Low	age (brassica-oleracea) seedlings caused by vapors of di butyl phthalate osure water, but unable to determine exact uptake route) Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
ration: > 21 days; Exposure Duration: > 21 days; Air; Not determined by study authors (i.e., che s Vascular Plants; <i>Brassica oleracea</i> ; Derby Da ent/Growth thalate (DBP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	ays emical of interest in expo y cultivar; Juvenile <u>Rating</u> Low Low	osure water, but unable to determine exact uptake route) Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Air; Not determined by study authors (i.e., che s Vascular Plants; <i>Brassica oleracea</i> ; Derby Da ent/Growth thalate (DBP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	emical of interest in expo y cultivar; Juvenile Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
y Vascular Plants; <i>Brassica oleracea</i> ; Derby Daent/Growth thalate (DBP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	y cultivar; Juvenile Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
y Vascular Plants; <i>Brassica oleracea</i> ; Derby Da ent/Growth thalate (DBP) <u>Metric</u> Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	y cultivar; Juvenile Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
ent/Growth thalate (DBP) Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Metric Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Rating Low Low	Comments It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Acclimatization and Pretreatment Conditions Number of Organisms and Replicates per Group	Low Low	It was not reported if any acclimation occurred. For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Conditions Number of Organisms and Replicates per Group	Low	For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Number of Organisms and Replicates per Group	Low	For each plastic tested, there were three replicates. In each cuvette, there were 4 plants per pot.
Replicates per Group		per pot.
		I I
A deguage of Test Conditions	Low	The electrony least of 200 during the day and 150 of a late but little other data its more
Adequacy of Test Conditions	Low	provided on environmental conditions.
Outcome Assessment Methodology	Low	It was reported the plants were visually assessed for signs of toxicity 4 weeks after sow- ing, but details regarding the assessment were not provided.
Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not
Assessment		reported. Plants were assessed for signs of toxicity, but this protocol was not described.
antrol		
	I	
Design and Procedures	Low	conditions or other non-treatment-related factors across study groups.
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.
alvsis		
Statistical Methods	Uninformative	Statistical analysis for this portion of the study was not conducted. Results were not presented in a manner that allowed for statistical analysis.
Reporting of Data	Medium	Results for the exposures were provided in Table 2, but negative control results were not reported.
Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.
	Outcome Assessment Methodology Consistency of Outcome Assessment ontrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure alysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes on of the evaluation was conducted in glass cuve	Outcome Assessment Methodology Low Consistency of Outcome Low Assessment Low ontrol Confounding Variables in Test Low Design and Procedures Outcomes Unrelated to Exposure Medium alysis Statistical Methods Uninformative Reporting of Data Medium Explanation of Unexpected Outcomes Low on of the evaluation was conducted in glass cuvettes. The test plastic was veral plastics were tested. It was reported that a control was run for each

toxicity as well. The study authors only tested for phthalates. Authors reported visually assessing the plants 1 month after sowing. Details regarding this protocol were not provided. The outcomes assessed for were mortality, growth, and chlorosis.

Overall Quality Determination

Uninformative

Study Citation:	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate						
Duration	emitted from	emitted from certain plastics. Annals of Applied Biology 105(1):97-105. Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route.	Terrestrial A	ir: Not determined by study authors (i e	chemical of interest in exposu	e water, but unable to determine exact untake route)			
Media. Path:	Terrestruit, T		, enemiear of merest in exposu				
Taxa, Species, Age:	Vegetation; V	ascular Plants; Brassica oleracea; Derby	y Day cultivar; Juvenile				
Health Outcome:	Mechanistic-	Photosynthesis	-				
Chemical:	Dibutyl phtha	alate (DBP)					
HERO ID:	5678863						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	e .		_				
	Metric 1:	Test Substance Identity	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP is the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.			
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	It was reported there was a negative control that contained zero plastic.			
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported. Several different plas- tics were reported to be testing, many of which had ND or a trace of DBP, but these did not appear to be the true negative control response.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.			
Domain 3: Exposure Cha	aracterization	Engening and Sectors /Test Media	T				
	Metric 7:	Preparation	Low	the test system was reported to be conducted in glass cuvettes containing 115mm diam- eter dwarf pots with four plants each. The plastic was suspended on a wire at the top of the cuvette that was ventilated. There was a non-plastic control for each type of plastic used. Measurements were taken of the DBP in the air. It is uncertain if the exposure was to DBP alone since other chemicals are involved in making plastic.			
	Metric 8:	Consistency of Exposure Administration	Medium	The exposure was reported to be for four weeks for all plants. All the plants were visu- ally assessed for one month post sowing. All seedlings were raised in 115mm diameter dwarf pots with four plants per pot.			
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be four weeks. This appeared adequate to observe the outcome of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, but to compare a glazing using DBP to a glazing using DIDP and to other plastics potentially plasticized with DBP.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solubility limit.			
Domain 4. Test Organism	n						
Domain 1. Test Organish	Metric 13:	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported.			
			Continued on next page				

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Environmental Hazard Evaluation

HERO ID: 5678863 Table: 3 of 6

		coi	ntinued from previous	page			
Study Citation:	Hardwick, F emitted fron Overall Dur	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Exposure Route, Media, Path:	Terrestrial; A						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Mechanistic Dibutyl phth 5678863	Vegetation; Vascular Plants; <i>Brassica oleracea</i> ; Derby Day cultivar; Juvenile Mechanistic-Photosynthesis Dibutyl phthalate (DBP) 5678863					
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	For each plastic tested, there were three replicates. In each cuvette, there were four plants per pot.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	The plants were kept at 20C during the day and 15C at night, but little other details were provided on environmental conditions.			
	Metric 17:	Outcome Assessment Methodology	Low	It was reported the plants were visually assessed for signs of toxicity four weeks after sowing, but details regarding the assessment were not provided.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. Plants were assessed for signs of toxicity, but this protocol was not described.			
Domain 6: Confoundir	ug / 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 Preser	ntation and Anal	lysis					
_ 511411 / Dum 110501	Metric 21:	Statistical Methods	Uninformative	Statistical analysis for this portion of the study was not conducted. Results were not presented in a manner that allowed for statistical analysis.			
	Metric 22:	Reporting of Data	Medium	Results for the exposures were provided in Table 2, but negative control results were not reported.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.			
Additional Comments:	This portion the cuvette.	of the evaluation was conducted in glass cuv Several plastics were tested. It was reported t	vettes. The test plastic v that a control was run fo	vas suspended from the top of the cuvette and chemicals vaporized into or each type of plastic, but control results were not reported. This study			

received an unacceptable ranking due to the lack of statistical analysis. There is also concern that other chemicals in the plastic could be vaporizing and causing toxicity as well. The study authors only tested for phthalates. Authors reported visually assessing the plants one month after sowing. Details regarding this protocol were not provided. The outcomes assessed for were mortality, growth, and chlorosis.

Overall Quality Determination

Dibutyl Phthalate

Uninformative

Study Citation:	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.						
Duration: Exposure Route, Media Path:	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; 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; Vascular Plants; <i>Brassica oleracea</i> ; Derby Day cultivar; Juvenile Development/Growth Dibutyl phthalate (DBP) 5678863 Metric Rating Comments						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP is the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.			
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported. The			
Domain 2: Test Design	Matria 4:	Nagatiya Controla	Uninformativa	For the average is the encentrouse only it did not average as they at an encouries.			
	Metric 4:	Negative Controls	Uninformative	Por the exposure in the greenhouse only, it did not appear as though an appropriate negative control was run concurrently with the test. Study authors reported growing plants in the original greenhouse with the DBP glazed strips and growing plants in the reglazed greenhouse, which reported the use of DIDP as the plasticizing agent. This is not a true chemical-free negative control.			
	Metric 5:	Negative Control Response	Uninformative	There was not a true negative control used in this study, so an appropriate negative con- trol response was not reported.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.			
Domain 2. Evenagues Ch	anastanization						
Domani 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	The test system was reported to be a glasshouse. One pot with 14 seedlings was placed in a compartment with the original glazing that contained DBP and another pot of 14 seedlings was placed in a compartment reglazed with DIDP. Little other details were provided about the test system.			
	Metric 8:	Consistency of Exposure Administration	Low	The exposure was reported to be for 4 weeks for all plants. However, according to Ta- ble 1, DBP levels were measured and appeared to be quite variable over time in the glasshouse. It is possible that exposure levels could have had a great deal of variation over the 4 week exposure period.			
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 16 weeks. This appeared adequate to observe the outcome of interest.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, to compare a glazing using DBP to a glazing using DIDP.			
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solubility limit.			

Domain 4: Test Organism

Continued on next page ...

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5678863 Table: 4 of 6

		col	ntinued from previous	page			
Study Citation:	Hardwick, R emitted from	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Media, Path:	X 7 / / · X						
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; <i>Brassica oleracea</i> ; Derby Day cultivar; Juvenile					
Chamical:	Developmen Dibutyl phth	Development/Orowin Dibutyl.nbthalate (DBP)					
HERO ID.	5678863	alate (DBI)					
Domain		Metric	Rating	Comments			
Domani	Metric 13.	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.			
		Conditions					
	Metric 15:	Number of Organisms and	Low	There was one pot with 14 plants for each treatment, which is lower than is typical.			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	The plants were kept at 20C during the day and 15C at night, but little other details were provided on environmental conditions.			
	Metric 17:	Outcome Assessment Methodology	Low	Plants were assessed for signs of toxicity at the end of the test, but this process was not described. No signs of toxicity were observed.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. Plats were assessed for signs of toxicity, but this protocol was not described.			
Domain & Confounding	Wariahla Car	atual					
Domain 6: Confounding	y Variable Col Metric 10:	Confounding Variables in Test	Low	The study did not provide anough information to allow a comparison of anyironmental			
	Wietric 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 Present	ation and Anal	vsis					
Domain 7. Data i lesent	Metric 21:	Statistical Methods	Uninformative	Statistical analysis for this portion of the study was not conducted			
	Metric 22:	Reporting of Data	Low	Results were reported in the text only for this portion of the study.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.			
Additional Comments:	This portion The other po control and c	of the evaluation was conducted in compartme t was grown in section that contained strips g lue to lack of statistics. The study authors rep	ents in a glasshouse. One lazed with DIDP. This s orted assessing the plant	e pot was grown in a section that contained glazed strips containing DBP. tudy received an unacceptable ranking due to the lack of a true negative is for signs of toxicity. This included mortality, growth, and chlorosis.			
Overall Qualit	ty Detern	nination	Uninformative	2			

Study Citation:	Hardwick, R	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Appals of Applied Biology 105(1):97-105						
Duration: Exposure Route, Media Path	Overall Dura Terrestrial; A	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; 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 Mortality Dibutyl phth 5678863	Vascular Plants; <i>Brassica oleracea</i> ; Derby D alate (DBP)	ay cultivar; Juvenile					
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP is the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.				
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported. The				
Domain 2: Test Design	Metric 4:	Negative Controls	Uninformative	For the exposure in the greenhouse only, it did not appear as though an appropriate negative control was run concurrently with the test. Study authors reported growing plants in the original greenhouse with the DBP glazed strips and growing plants in the				
	Metric 5:	Negative Control Response	Uninformative	reglazed greenhouse, which reported the use of DIDP as the plasticizing agent. This is not a true chemical-free negative control. There was not a true negative control used in this study, so an appropriate negative con-				
				trol response was not reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.				
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media Preparation	Low	The test system was reported to be a glasshouse. One pot with 14 seedlings was placed in a compartment with the original glazing that contained DBP and another pot of 14 seedlings was placed in a compartment reglazed with DIDP. Little other details were provided about the test system				
	Metric 8:	Consistency of Exposure Administration	Low	The exposure was reported to be for 4 weeks for all plants. However, according to Ta- ble 1, DBP levels were measured and appeared to be quite variable over time in the glasshouse. It is possible that exposure levels could have had a great deal of variation over the 4 week exposure period.				
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 16 weeks. This appeared adequate to observe the outcome of interest.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, to compare a glazing using DBP to a glazing using DIDP.				
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solubility limit.				

Domain 4: Test Organism

Continued on next page ...

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5678863 Table: 5 of 6

		•••• CO	ntinued from previous	page
Study Citation:	Hardwick, R emitted from	R. C., Cole, R. A., Fyfield, T. P. (1984). Injust certain plastics, Annals of Applied Biology	ry to and death of cabba 105(1):97-105	age (brassica-oleracea) seedlings caused by vapors of di butyl phthalate
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21 days	avs	
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e., che	emical of interest in expo	osure water, but unable to determine exact uptake route)
Media. Path:	,-	,		······································
Taxa Species Age	Vegetation [.]	Vascular Plants: <i>Brassica oleracea</i> : Derby Da	v cultivar: Iuvenile	
Health Outcome	Mortality	vasediai Thanks, Brassiea overacea, Deroy Da	y cultival, su chile	
Chamical:	Dibutyl phth	alate (DRD)		
	5678863			
IIERO ID.	3078803			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.
	Matria 15.	Conditions	Low	
	Metric 15:	Number of Organisms and	Low	There was one pot with 14 plants for each treatment, which is lower than is typical.
		Replicates per Group		
Domain 5: Outcome As	sessment			
Domain 5. Outcome 713	Metric 16.	Adequacy of Test Conditions	Low	The plants were kept at 20C during the day and 15C at night, but little other details were
	Methe 10.	Adequacy of Test Conditions	Low	provided on environmental conditions.
	Metric 17:	Outcome Assessment Methodology	Low	Plants were assessed for signs of toxicity at the end of the test, but this process was not
		8,		described. No signs of toxicity were observed.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not
		Assessment		reported. Plats were assessed for signs of toxicity, but this protocol was not described.
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	ysis		
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis for this portion of the study was not conducted.
	Metric 22:	Reporting of Data	Low	Results were reported in the text only for this portion of the study.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.
Additional Comments:	This portion The other po control and o	of the evaluation was conducted in compartm of was grown in section that contained strips g due to lack of statistics. The study authors rep	ents in a glasshouse. On glazed with DIDP. This s ported assessing the plant	e pot was grown in a section that contained glazed strips containing DBP. study received an unacceptable ranking due to the lack of a true negative ts for signs of toxicity. This included mortality, growth, and chlorosis.
Overall Qualit	ty Detern	nination	Uninformative	e

Study Citation:	Hardwick, R emitted from	Hardwick, R. C., Cole, R. A., Fyfield, T. P. (1984). Injury to and death of cabbage (brassica-oleracea) seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Annals of Applied Biology 105(1):97-105.						
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; A	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; 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 Mechanistic- Dibutyl phth 5678863	Vascular Plants; <i>Brassica oleracea</i> ; Derby D Photosynthesis alate (DBP)	ay cultivar; Juvenile					
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce Metric 1:	Test Substance Identity	Low	The DBP originated from glazing strips in a greenhouse. DBP was measured in the greenhouse or in the cuvettes during the experiment. However, it is possible there was contamination from substances other than DBP in the glazing strips. DBP was reported to be used as a plasticizer for the strips, but other chemical exposure cannot be ruled out.				
	Metric 2:	Test Substance Source	Low	The source of the glazing strip was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.				
Domain 2: Test Design	Matric 1:	Nagativa Controls	Uninformative	For the averagers in the greenhouse only, it did not encourses though an engraprists				
	Metric 4.	Negative Controls	Chinionnauve	rot the exposure in the greenhouse only, it the hot appear as though an appropriate negative control was run concurrently with the test. Study authors reported growing plants in the original greenhouse with the DBP glazed strips and growing plants in the reglazed greenhouse, which reported the use of DIDP as the plasticizing agent. This is not a true chemical-free negative control.				
	Metric 5:	Negative Control Response	Uninformative	There was not a true negative control used in this study, so an appropriate negative con- trol response was not reported.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the plants were allocated into study groups.				
	, . <i>.</i> .							
Domain 5: Exposure Cn	Metric 7:	Experimental System/Test Media Preparation	Low	The test system was reported to be a glasshouse. One pot with 14 seedlings was placed in a compartment with the original glazing that contained DBP and another pot of 14 seedlings was placed in a compartment reglazed with DIDP. Little other details were provided about the test system.				
	Metric 8:	Consistency of Exposure Administration	Low	The exposure was reported to be for four weeks for all plants. However, according to Table 1, DBP levels were measured and appeared to be quite variable over time in the glasshouse. It is possible that exposure levels could have had a great deal of variation over the 4-week exposure period.				
	Metric 9:	Measurement of Test Substance Concentration	High	Samples of air from the test environment were measured using gas chromatography to test for various phthalates.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 16 weeks. This appeared adequate to observe the outcome of interest.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The purpose of the study was not to observe a dose response, to compare a glazing using DBP to a glazing using DIDP.				
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations measured using gas chromatography were below the water solubility limit.				

Domain 4: Test Organism

Continued on next page ...

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5678863 Table: 6 of 6

		co	ntinued from previous	page
Study Citation:	Hardwick, R emitted from	R. C., Cole, R. A., Fyfield, T. P. (1984). Inju	ry to and death of cabba 105(1):97-105	ge (brassica-oleracea) seedlings caused by vapors of di butyl phthalate
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21 d	avs	
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e., cho	emical of interest in expo	osure water, but unable to determine exact uptake route)
Media. Path:	,-			······································
Taxa, Species, Age:	Vegetation:	Vascular Plants: <i>Brassica oleracea</i> : Derby Da	ov cultivar: Juvenile	
Health Outcome:	Mechanistic	-Photosynthesis	y calification, calification	
Chemical:	Dibutyl phth	palate (DBP)		
HERO ID:	5678863			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	The cabbage was reported to be the Derby Day cultivar, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.
	Metric 15:	Conditions Number of Organisms and	Low	There was one not with 14 plants for each treatment, which is lower than is twnical
	Mettre 15.	Replicates per Group	LOW	There was one pot with 14 plants for each treatment, which is lower than is typical.
		Replicates per Gloup		
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	The plants were kept at 20C during the day and 15C at night, but little other details were
		1		provided on environmental conditions.
	Metric 17:	Outcome Assessment Methodology	Low	Plants were assessed for signs of toxicity at the end of the test, but this process was not
				described. No signs of toxicity were observed.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not
		Assessment		reported. Plants were assessed for signs of toxicity, but this protocol was not described.
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 Pro-	otion and Arr-1	husia		
Domain /: Data Present	Motrio 21.	19818 Statistical Methods	Uninformativa	Statistical analysis for this partian of the study was not conducted
	Metric 21:	Statistical Methods Deporting of Data	Law	Statistical analysis for this portion of the study was not conducted.
	Metric 22:	Explored on Data	Low	Results were reported in the text only for this portion of the study.
	Metric 23:	Explanation of Unexpected Outcomes	Low	outcomes occurred.
Additional Comments:	This portion	of the evaluation was conducted in compartm	ents in a glasshouse. On	e pot was grown in a section that contained glazed strips containing DBP.
	The other po	ot was grown in section that contained strips g	glazed with DIDP. This s	tudy received an unacceptable ranking due to the lack of a true negative
	control and o	due to lack of statistics. The study authors rep	ported assessing the plant	ts for signs of toxicity. This included mortality, growth, and chlorosis.
		• • • •	TT • • •	
Overall Qualit	ty Detern	nination	Uninformative	

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. physiologica Journal of A Overall Dura Terrestrial; V Vegetation; V Mechanistic Dibutyl phth 5043543	 Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Water; Root uptake Vegetation; Vascular Plants; <i>Brassica parachinensis</i>; Huaguan; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Cytotoxicity-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 5043543 				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice					
	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 or grade of test substance 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 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	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	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 and exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	A suitable number of treatments over an adequate range of concentrations was reported		
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All test concentrations exceeded the water solubility value of 13 mg/L		
Domain 4: Test Organis	sm					
-	Metric 13:	Test Organism Characteristics	Low	The source of the test plants 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	Low	Initial number not reported. 5 roots were pooled per sampling in triplicate		
		Replicates per Group				

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5043543 Table: 1 of 4

		0	continued from previous pa	ge		
Study Citation:	Zhao, H. M. physiologica Journal of A	Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551.				
Duration:	Overall Dura	tion: 11 - 21 days; Exposure Duration: 11 -	- 21 days			
Exposure Route,	Terrestrial; V	Vater; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation; Vascular Plants; Brassica parachinensis; Huaguan; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Cytotoxicity-Oxidative stress (including redox biology)					
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5043543					
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: 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 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 for every measurement, but results were described in the text, figures and charts		
	Metric 23:	Explanation of Unexpected Outcomes	Low	insufficient information was provided to determine if unexpected outcomes occurred		
Additional Comments:	None					
Overall Qualit	ty Detern	nination	Uninformative			

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	 Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Water; Root uptake Vegetation; Vascular Plants; <i>Brassica parachinensis</i>; Lvbao; Not Applicable (e.g., fungi or algae studies) or Not Reported 						
Health Outcome: Chemical:	Developmen Dibutyl phth	Development/Growth Dibutyl phthalate (DBP)					
HERO ID:	5043543						
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	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.			
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	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	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 and exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	A suitable number of treatments over an adequate range of concentrations was reported.			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All test concentrations exceeded the water solubility value of 13 mg/L.			
Domain 4: Test Organis	m						
2	Metric 13:	Test Organism Characteristics	Low	The source of the test plants 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	Low	The initial number of organisms was not reported. Five roots were pooled per sampling in triplicate.			
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.			
		С	continued on next page .				

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Dibutyl Phthalate

		con	tinued from previou	s page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Zhao, H. M. physiologica Journal of A Overall Dura Terrestrial; V Vegetation; V	, Huang, H. B., Luo, Y. M., Huang, C. Q., D and proteomic responses to dibutyl phthalate gricultural and Food Chemistry 66(51):13541- ation: 11 - 21 days; Exposure Duration: 11 - 21 Water; Root uptake Vascular Plants; <i>Brassica parachinensis</i> ; Lvbac	u, H., Xiang, L., Cai (DBP) exposure betw 13551. days o; Not Applicable (e.g	, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root reen low- and high-DBP accumulation cultivars of Brassica parachinensis.	
Health Outcome:	Development/Growth				
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	5043543				
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 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.	
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	Low	Data for exposure-related findings were not shown for each treatment and control group for every measurement, but results were described in the text.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	There was insufficient information provided to determine if unexpected outcomes oc- curred.	
Additional Comments:	None				

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path:	Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Water; Root uptake						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V Mechanistic Dibutyl phth 5043543	egetation; Vascular Plants; <i>Brassica parachinensis</i> ; Lvbao; Not Applicable (e.g., fungi or algae studies) or Not Reported techanistic-Biomarkers (exposure and effect)-Cell signaling/function-Cytotoxicity-Oxidative stress (including redox biology) ibutyl phthalate (DBP) 043543					
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 or grade of test substance 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 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	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 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 exposure frequency were reported and appropriate for the study type			
	Metric 11:	Number of Exposure Groups/	High	A suitable number of treatments over an adequate range of concentrations was reported			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All test concentrations exceeded the water solubility value of 13 mg/L			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported			
	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	Initial number not reported. 5 roots were pooled per sampling in triplicate			
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	High	The outcome assessment methodology reported the intended outcome of interest			
		(Continued on next page				

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Environmental Hazard Evaluation

HERO ID: 5043543 Table: 3 of 4

		con	ntinued from previous	page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M., physiological Journal of Ag Overall Dura Terrestrial; W Vegetation; V Mechanistic- Dibutyl phtha 5043543	 Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Water; Root uptake Vegetation; Vascular Plants; <i>Brassica parachinensis</i>; Lvbao; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Cytotoxicity-Oxidative stress (including redox biology) Dibutyl phthalate (DBP) 5043543 				
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 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 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 not shown for each treatment and control group for every measurement, but results were described in the text, figures and charts		
	Metric 23:	Explanation of Unexpected Outcomes	Low	insufficient information was provided to determine if unexpected outcomes occurred		
Additional Comments:	None					

Overall Quality Determination

Uninformative

Dibutyl Phthalate

Study Citation:Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Different physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassical Journal of Agricultural and Food Chemistry 66(51):13541-13551.Duration:Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 daysTerrestrial; Water; Root uptake				Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root en low- and high-DBP accumulation cultivars of Brassica parachinensis.			
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Developmen Dibutyl phth 5043543	Vegetation; Vascular Plants; <i>Brassica parachinensis</i> ; Huaguan; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 5043543					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	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 or grade of test substance 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	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	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			
	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	High	A suitable number of treatments over an adequate range of concentrations was reported			
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All test concentrations exceeded the water solubility value of 13 mg/L			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants 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	Low	Initial number not reported. 5 roots were pooled per sampling in triplicate			
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			
		С	continued on next page .				

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PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Dibutyl Phthalate

		con	tinued from previou	s page			
Study Citation: Duration:	Zhao, H. M. physiologica Journal of A Overall Dura	Zhao, H. M., Huang, H. B., Luo, Y. M., Huang, C. Q., Du, H., Xiang, L., Cai, Q. Y., Li, Y. W., Li, H., Mo, C. H., He, Z. (2018). Differences in root physiological and proteomic responses to dibutyl phthalate (DBP) exposure between low- and high-DBP accumulation cultivars of Brassica parachinensis. Journal of Agricultural and Food Chemistry 66(51):13541-13551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; V	Water; Root uptake					
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Brassica parachinensis; Huaguan; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	5043543						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported			
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			
	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					
Domain 7. Dua 11050	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 for every measurement, but results were described in the text			

Overall Quality Determination

Uninformative

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; V Mechanistic Dibutyl phth	 Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L.</i>; Lvbao70 cultivar; Embryo Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 					
HERO ID:	3070947	3070947					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	Trat Caletan a Idaatita	τ	ו ו י ו מתרו ש			
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	High	The DBP was identified by name only. The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3 and in Figures 2-4. The control response was adequate for the outcomes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Hıgh	Soil was spiked with the appropriate amount of DBP dissolved in acetone. The acetone was allowed to evaporate and the soil was aged for 2 weeks before the start of the study. Exposure was conducted in ceramic pots.			
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. seedlings were thinned to 5 seedlings per pot at 15d and watered daily.			
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.			
	Metric 11:	Number of Exposure Groups/	Low	There were only 2 exposure groups, which is quite a bit lower than normal. This was still adequate to see a response			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.			
	· ·	<u> </u>		<u> </u>			
Domain 4: Test Organis	m						
-	Metric 13:	Test Organism Characteristics	Low	The source of the B. parachinensis L. was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.			
	Metric 15:	Conditions Number of Organisms and	Medium	There were 5 seedlings per test chamber. Each exposure was performed in triplicate.			
		Replicates per Group					
		Conti	nued on next pa	ge			

Environmental Hazard Evaluation

HERO ID: 3070947 Table: 1 of 6

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; Mechanistic	, Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q BP) exposure between low- and high-DBP a 8(Pt B):840-849. ation: > 21 days; Exposure Duration: > 21 c Soil; Not determined by study authors (i.e., cl Vascular Plants; <i>Brassica parachinensis L</i> .; L -Oxidative stress (including redox biology)-F	. Y., Mo, C. H., accumulating cu days hemical of inter _vbao70 cultivat Photosynthesis	Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl litivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental est in exposure water, but unable to determine exact uptake route) ;; Embryo	
Chemical: HERO ID:	Dibutyl phthalate (DBP) 3070947 Metric Rating Comments				
Domain					
Domain 5: Outcome As	sessment				
2	Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in chlorophyll, and changes in SOD, CAT, GST, and MDA.	
	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.	
Domain 7: Data Present	ation and Anal	vsis			
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section and were appro- priate for the study.	
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in Table 3 and in Figures 2-4.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures and tables.	
Additional Comments:	This potion mechanistic	of the evaluation was on the effect of DBP outcomes of photosynthesis and oxidative st	on chlorophyll ress were chose	a, SOD, CAT, GST, and MDA the Lvbao70 cultivar of B parachinensis L. The n as the outcomes of interest.	
Overall Qualit	ty Deterr	nination	Medium		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; ¹ ADME (biot Dibutyl phth 3070947	Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L</i> .; Huaguan cultivar; Embryo ADME (biotransformation) Dibutyl phthalate (DBP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure S1a. It was appropriate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.		
Domain 2. Europuna Ch	matarization					
Domain 5. Exposure Cha	Metric 7:	Experimental System/Test Media	High	Soil was spiked with the appropriate amount of DBP dissolved in acetone. The acetone		
		Preparation		Exposure was conducted in ceramic pots.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. seedlings were thinned to 5 seedlings per pot at 15d and watered daily.		
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/	Low	There were only 2 exposure groups, which is quite a bit lower than normal. This was still adequate to see a response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.		
Domain 4: Test Organisr	II Matria 12:	Test Organism Characteristics	Low	The course of the D perception of I was not reported		
	Metric 14:	Acclimatization and Protreatment	Low	It was not reported if the argonisms ware applied in any way		
	wicult 14:	Conditions	LOW	it was not reported if the organisms were acclimated in any way.		
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 5 seedlings per test chamber. Each exposure was performed in triplicate.		

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 3070947 Table: 2 of 6

		contin	ued from previ	ious page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L.</i>; Huaguan cultivar; Embryo ADME (biotransformation) Dibutyl phthalate (DBP) 3070947 				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DBP accumulation in the roots and shoots.	
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups–DBP accumulation was determined by GC-MS.	
Domain 6: Confounding	y / Variable Co	ntrol			
2 onian of Companying	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 methods were reported in the "Statistical Analysis" section and were appropriate for the study.	
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in the supplemen- tal figure 1a and was appropriate for the outcomes of interest.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figure.	
Additional Comments:	This potion outcome of i	of the evaluation was on the accumulation of the evaluation of the	of DBP in the sh	noots and roots of B. parachinensis cultivar Huaguan. ADME was selected as the	
Overall Qualit	ty Detern	nination	Medium		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; Developmen Dibutyl phth 3070947	 Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl obthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. Overall Duration: > 21 days; Exposure Duration: > 21 days Ferrestrial; 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>Brassica parachinensis L.</i>; Lvbao70 cultivar; Embryo Development/Growth Dibutyl phthalate (DBP) 				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure S1b and in Figure 1. It was appro- priate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.		
	, . ,.					
Domain 3: Exposure Ch	aracterization		TT' 1			
	Metric 7:	Preparation	High	soil was spiked with the appropriate amount of DBP dissolved in acetone. The acetone was allowed to evaporate and the soil was aged for 2 weeks before the start of the study. Exposure was conducted in ceramic pots.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. seedlings were thinned to 5 seedlings per pot at 15d and watered daily.		
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups, which is quite a bit lower than normal. This was still adequate to see a response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the B. parachinensis L. was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 5 seedlings per test chamber. Each exposure was performed in triplicate.		

Domain 5: Outcome Assessment

Continued on next page ...
Environmental Hazard Evaluation

HERO ID: 3070947 Table: 3 of 6

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L</i> .; Lvbao70 cultivar; Embryo Development/Growth Dibutyl phthalate (DBP) 3070047				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-plant biomass and changes in structure.	
	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 end of the study, plants were divided into roots and shoots, and fresh weights were recorded. Plant tissues were also examined via TEM to assess leaf ultrastructure.	
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	lysis			
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section and were appropriate for the study.	
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in the supplemen- tal figure 1b and was appropriate for the outcomes of interest. Leaf structure TEM was provided in Figure 1 for both treatments and controls.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figure.	
Additional Comments:	This potion growth was	of the evaluation was on the effect of DBF selected as the outcome of interest.	on plant biom	ass and leaf structure of B. parachinensis L cultivar Lvbao70. Development and	
Overall Qualit	ty Deterr	nination	Medium		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; Y Developmen Dibutyl phth 3070947	Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L.</i> ; Huaguan cultivar; Embryo Development/Growth Dibutyl phthalate (DBP) 3070947				
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure S1b and in Figure 1. It was appro- priate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.		
Damain 2. Ennamer Ch						
Domain 3: Exposure Ch	Matria 7	Experimental System/Test Madia	Iliah			
	Metric 7:	Preparation	High	soil was spiked with the appropriate amount of DBP dissolved in acetone. The acetone was allowed to evaporate and the soil was aged for 2 weeks before the start of the study. Exposure was conducted in ceramic pots.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. seedlings were thinned to 5 seedlings per pot at 15d and watered daily.		
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups, which is quite a bit lower than normal. This was still adequate to see a response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.		
		- •				
Domain 4: Test Organisi	n					
	Metric 13:	Test Organism Characteristics	Low	The source of the B. parachinensis L. was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 5 seedlings per test chamber. Each exposure was performed in triplicate.		
		Repleates per Group				

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 3070947 Table: 4 of 6

		contin	ued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-buty phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmenta Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L.</i>; Huaguan cultivar; Embryo Development/Growth Dibutyl phthalate (DBP) 3070947 				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant biomass and changes in structure.	
	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 end of the study, plants were divided into roots and shoots, and fresh weights were recorded. Plant tissues were also examined via TEM to assess leaf ultrastructure.	
Domain 6: Confounding	y / Variable Co	ntrol			
Domain of Comountaing	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	lvsis			
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section and were appropriate for the study.	
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in the supplemen- tal figure 1b and was appropriate for the outcomes of interest. Leaf structure TEM was provided in Figure 1 for both treatments and controls.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figure.	
Additional Comments:	This potion growth was	of the evaluation was on the effect of DBP selected as the outcome of interest.	on plant bioma	ass and leaf structure of B. parachinensis L cultivar Huaguan. Development and	
Overall Quali	ty Deterr	nination	Medium		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. phthalate (D Pollution 200 Overall Dura Terrestrial; S Vegetation; V ADME (biot Dibutyl phth 3070947	Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849. 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) Vegetation; Vascular Plants; <i>Brassica parachinensis L.</i> ; Lvbao70 cultivar; Embryo ADME (biotransformation) Dibutyl phthalate (DBP) 3070947				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure S1a. It was appropriate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.		
	, . <u>.</u> .					
Domain 3: Exposure Ch	aracterization	Environmental Scottant/Teach Madia	TT: -1-	י די די די אור אין אין אין אין אין אין אין אין א		
	Metric 7:	Preparation	High	soil was spiked with the appropriate amount of DBP dissolved in acetone. The acetone was allowed to evaporate and the soil was aged for 2 weeks before the start of the study. Exposure was conducted in ceramic pots.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. seedlings were thinned to 5 seedlings per pot at 15d and watered daily.		
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups, which is lower than normal. This was still adequate to see a response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.		
		· · · ·				
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the B. parachinensis L. was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.		
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were 5 seedlings per test chamber. Each exposure was performed in triplicate.		

Domain 5: Outcome Assessment

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Environmental Hazard Evaluation

HERO ID: 3070947 Table: 5 of 6

		contin	ued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Zhao, H. M. phthalate (D Pollution 20 Overall Dura Terrestrial; S Vegetation; ' ADME (biot Dibutyl phth 3070947	, Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q BP) exposure between low- and high-DBP a 8(Pt B):840-849. ation: > 21 days; Exposure Duration: > 21 d Soil; Not determined by study authors (i.e., c Vascular Plants; <i>Brassica parachinensis L</i> .; I transformation) halate (DBP)	9. Y., Mo, C. H., accumulating cu days hemical of inter Lvbao70 cultiva	Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl lltivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental est in exposure water, but unable to determine exact uptake route) r; Embryo
Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DBP accumulation in the roots and shoots.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups–DBP accumulation was determined by GC-MS.
Domain 6: Confounding	y / Variable Co	ntrol		
2 onium of Comountaine	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 methods were reported in the "Statistical Analysis" section and were appropriate for the study.
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in the supplemen- tal figure 1a and was appropriate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figure.
Additional Comments:	This potion outcome of i	of the evaluation was on the accumulation conterest.	of DBP in the sh	noots and roots of B. parachinensis cultivar Lvbao70. ADME was selected as the
Overall Qualit	ty Detern	nination	Medium	

Study Citation:	Zhao, H. M. phthalate (D Pollution 20	Zhao, H. M., Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q. Y., Mo, C. H., Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-butyl phthalate (DBP) exposure between low- and high-DBP accumulating cultivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmental Pollution 208(Pt B):840-849.				
Duration: Exposure Route, Modio, Both	Overall Dura Terrestrial; S	ation: > 21 days; Exposure Duration: > 21 Soil; Not determined by study authors (i.e., c	est in exposure water, but unable to determine exact uptake route)			
Media, Path: Taya Species Age:	Vegetation:	Vascular Plants: Brassica parachinensis I ·]	Huaguan cultiva	r: Embryo		
Health Outcome:	Mechanistic	-Oxidative stress (including redox biology)-	Photosvnthesis	, Energe		
Chemical:	Dibutyl phth	alate (DBP)	j i i i			
HERO ID:	3070947					
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Aladdin Chemistry Co. Ltd. in China, and DBP was verified by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	Study authors reported using soil that was not spiked with DBP as a negative control.		
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3 and in Figures 2-4. The control response was adequate for the outcomes of interest.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.		
Domain 2. Europure Ch	anastanization					
Domain 5: Exposure Ch	Metric 7:	Experimental System/Test Media	High	Sail was spiked with the appropriate amount of DPD dissolved in sectors. The sectors		
	Weute 7.	Preparation	Ingn	was allowed to evaporate, and the soil was aged for two weeks before the start of the study. Exposure was conducted in ceramic pots.		
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for 45d in ceramic pots that were 20cm in diameter and 14cm in height in a greenhouse with natural lighting conditions. Seedlings were thinned to five per pot at 15d and watered daily.		
	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:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be for 45 days. This was adequate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/	Low	There were only two exposure groups, which is quite a bit lower than normal. This was still adequate to see a response		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.		
				•		
Domain 4: Test Organisi	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the B. parachinensis L. was not reported.		
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated in any way.		
	Metric 15:	Conditions Number of Organisms and Peplicates per Group	Medium	There were five seedlings per test chamber. Each exposure was performed in triplicate.		
		Replicates per Group				

Domain 5: Outcome Assessment

Continued on next page ...

Environmental Hazard Evaluation

HERO ID: 3070947 Table: 6 of 6

Zhao, H. M., phthalate (Dl Pollution 208 Overall Dura Terrestrial; S Vegetation; V	Du, H., Xiang, L., Li, Y. W., Li, H., Cai, Q BP) exposure between low- and high-DBP a 3(Pt B):840-849. tion: > 21 days; Exposure Duration: > 21 d oil; Not determined by study authors (i.e., c Vascular Plants; <i>Brassica parachinensis L</i> ; F	. Y., Mo, C. H., accumulating cu days hemical of inter Huaguan cultiva	Cao, G., Wong, M. H. (2016). Physiological differences in response to di-n-buty iltivars of Chinese flowering cabbage (Brassica parachinensis L.). Environmenta est in exposure water, but unable to determine exact uptake route) r; Embryo
Mechanistic- Dibutyl phth	Oxidative stress (including redox biology)-I alate (DBP)	Photosynthesis	
3070947			
	Metric	Rating	Comments
Metric 16:	Adequacy of Test Conditions	Low	It was reported that natural light was used in the greenhouse, but it was not reported what this was. Temperature was reported to be 25-32C. It was unclear if plants were fertilized more than once. Relative humidity was not reported.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in chlorophyll, and changes in SOD, CAT, GST, and MDA.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Variable Cor	atrol		
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.
ion and Anal	vsis		
Metric 21:	Statistical Methods	High	Statistical methods were reported in the "Statistical Analysis" section and were appropriate for the study.
Metric 22:	Reporting of Data	High	Data for the exposure response and the control response was reported in Table 3 and in Figures 2-4.
Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures and tables.
This potion of mechanistic of	of the evaluation was on the effect of DBP outcomes of photosynthesis and oxidative st	on chlorophyll ress were chose	a, SOD, CAT, GST, and MDA the Huaguan cultivar of B parachinensis L. The n as the outcomes of interest.
	phthalate (D) Pollution 208 Overall Dura Terrestrial; S Vegetation; V Mechanistic- Dibutyl phth 3070947 Metric 16: Metric 17: Metric 17: Metric 18: Variable Cor Metric 19: Metric 20: ion and Analy Metric 21: Metric 22: Metric 23: This potion of	phthalate (DBP) exposure between low- and high-DBP a Pollution 208(Pt B):840-849. Overall Duration: > 21 days; Exposure Duration: > 21 of Terrestrial; Soil; Not determined by study authors (i.e., c Vegetation; Vascular Plants; <i>Brassica parachinensis L</i> .; I Mechanistic-Oxidative stress (including redox biology)-1 Dibutyl phthalate (DBP) 3070947 <u>Metric</u> Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome Assessment Variable Control Metric 20: Outcomes Unrelated to Exposure ion and Analysis Metric 21: Statistical Methods Metric 22: Reporting of Data Metric 23: Explanation of Unexpected Outcomes This potion of the evaluation was on the effect of DBP mechanistic outcomes of photosynthesis and oxidative st	phthalate (DBP) exposure between low- and high-DBP accumulating ct Pollution 208(Pt B):840-849. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Not determined by study authors (i.e., chemical of inter Vegetation; Vascular Plants; <i>Brassica parachinensis L</i> .; Huaguan cultiva Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 3070947 Metric Rating Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Methodology Metric 18: Consistency of Outcome Metric 19: Confounding Variables in Test Low Design and Procedures Metric 20: Outcomes Unrelated to Exposure Metric 21: Statistical Methods Metric 22: Reporting of Data Metric 23: Explanation of Unexpected Outcomes High

Study Citation:	Liao, C. S.,	Yen, J. H., Wang, Y. S. (2009). Growth inh	nibition in Chines	se cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate.			
Duration	Journal of H	azardous Materials $163(2-3):625-631$.	dave				
Exposure Route.	Terrestrial: V	Vater: Not determined by study authors (i.e.	chemical of int	erest in exposure water, but unable to determine exact uptake route)			
Media, Path:	10110301101, I		., •• 01				
Taxa, Species, Age:	Vegetation; V	legetation; Vascular Plants; Brassica rapa; var. chinensis; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ADME (biotransformation)					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1296241						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	DBP was identified by CAS number.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Riedel-deHaen Co. in Germany, but it was not reported if it was analytically verified.			
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate solvent control.			
	Metric 5:	Negative Control Response	High	The negative control response was adequate and was reported in Tables 1 and 3.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the cabbage was allocated into study groups.			
Domain 3: Exposure Ch	aracterization		т				
	Metric /:	Preparation	Low	DBP was added to acetone at a concentration of 100g/L to create the stock solution. It was not reported how the test concentrations were prepared. It was also not reported how often new test solution was added to the system. The type of experimental vessel (plastic or glass) used was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	All test exposures were conducted in 3L pots with 2.5L of solution at the same tempera- ture and relative humidity. Photoperiod was 16L:8D with 25C during the day and 20C at night. It was not reported how often the test solution was replaced.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.			
	Metric 10:	Exposure Duration and Frequency	High	The duration of the study was reported to be 42d. This was adequate to observe a re- sponse.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Study authors reported four study groups plus a solvent control. This was adequate for the outcomes of interest.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent. The solvent control response was appropriate.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the cabbage seeds was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the plants were acclimated in any way to test conditions.			
	Metric 15:	Conditions Number of Organisms and	Low	The number of test organisms in each replicate was not reported. It was reported that			
		Replicates per Group		tests were carried out in triplicate.			
		Conti	nued on next pa	ge			

Environmental Hazard Evaluation

HERO ID: 1296241 Table: 1 of 3

		contin	ued from previ	ous page			
Study Citation:	Liao, C. S., Journal of H	Liao, C. S., Yen, J. H., Wang, Y. S. (2009). Growth inhibition in Chinese cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate. Journal of Hazardous Materials 163(2-3):625-631.					
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	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:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica rapa; var. chinensi	is; Not Applicab	le (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (bio	transformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1296241						
Domain		Metric	Rating	Comments			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Authors reported growing the organisms in a solution adapted from Hoagland's solution.			
		1 2	C	There was 2.5L of media in each pot. Organisms were grown at 25C during the day and 20C at night with a 16L:8D photoperiod. Relative humidity was reported to be 70-95%.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-accumulation of DBP in plant tissue.			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed			
		Assessment		consistently across study groups. The assessment process was discussed in detail in section 2.4.			
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. It was not re- ported if the plants 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 Present	ation and Anal	veic					
Domain 7. Data i lesent	Metric 21.	Statistical Methods	High	T-tests were used to analyze data			
	Metric 22:	Reporting of Data	High	All accumulation data was reported in Table 1 along with the control response. Data was appropriate for the outcome of interest. Table 3 also had a summary of the data.			
	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 as the outcome	of the evaluation was on the accumulation o me of interest.	of DBP in Chine	se cabbage after a 42-day exposure at various concentrations. ADME was selected			
Overall Onali	tv Deterr	nination	Medium				
- · · · · · · · · · · · · · · · · · · ·							

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Liao, C. S., Yen, J. H., Wang, Y. S. (2009). Growth inhibition in Chinese cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate.
	Journal of Hazardous Materials 163(2-3):625-631.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route,	Terrestrial; 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; Brassica rapa; var. chinensis; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	1296241

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	DBP was identified by CAS number.
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Riedel-deHaen Co. in Germany, but it was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate solvent control.
	Metric 5:	Negative Control Response	High	The negative control response was adequate and was reported in Table 2 for chlorophyll and in Figures 3 and 4 for the proteomic portion of the study.
	Metric 6:	Randomized Allocation	Low	It was not reported how the cabbage was allocated into study groups.
Domain 3: Exposure Char	acterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	DBP was added to acetone at a concentration of 100g/L to create the stock solution. It was not reported how the test concentrations were prepared. It was also not reported how often new test solution was added to the system. The type of experimental vessel (glass or plastic) used was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	All test exposures were conducted in 3L pots with 2.5L of solution at the same tempera- ture and relative humidity. Photoperiod was 16L:8D with 25C during the day and 20C at night. It was not reported how often the test solution was replaced.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the study was reported to be 42d. This was adequate to observe a re- sponse.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Study authors reported 4 study groups plus a solvent control. This was adequate for the outcomes of interest.
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent. The solvent control response was appropriate.
Domain 4: Test Organism				
C	Metric 13:	Test Organism Characteristics	Low	The source of the cabbage seeds was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the plants were acclimated in any way to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms in each replicate was not reported. It was reported that tests were carried out in triplicate.
		Conti	nued on next pa	nge

Environmental Hazard Evaluation

HERO ID: 1296241 Table: 2 of 3

		contin	ued from previ	ous page		
Study Citation:	Liao, C. S., Journal of H	Yen, J. H., Wang, Y. S. (2009). Growth inhiazardous Materials 163(2-3):625-631.	ibition in Chine	se cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate.		
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days			
Exposure Route,	Terrestrial; V	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:	Vegetation; Y	Vascular Plants; Brassica rapa; var. chinensi	is; Not Applicab	le (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosyn	thesis			
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1296241					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Authors reported growing the organisms in a solution adapted from Hoagland's solution. There was 2.5L of media in each pot. Organisms were grown at 25C during the day and 20C at night with a 16L:8D photoperiod. Relative humidity was reported to be 70-95%.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–chlorophyll a and b concentration and protein expression.		
	Metric 18:	Consistency of Outcome Assessment	High	Details concerning the proteomics assessment portion of the study were reported in detail in section 2.5.		
Domain 6: Confounding	a / Variable Co	ntrol				
Domain 0. Comountaing	Metric 19.	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. It was not re- ported if the plants 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 Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	T-tests were used to analyze data.		
	Metric 22:	Reporting of Data	High	Data was reported in Table 2 for chlorophyll concentrations and in Figures 3 and 4 and Table 4 for protein expression. Control data was reported for both mechanistic outcomes.		
	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 photosynthe	of the evaluation was on the effect of Dl sis and biomarker mechanistic outcomes we	BP on chloroph re chosen as the	yll a and b concentration and on protein expression in Chinese cabbage. The outcomes of interest.		

Overall Quality Determination

Medium

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Liao, C. S., Yen, J. H., Wang, Y. S. (2009). Growth inhibition in Chinese cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate.
	Journal of Hazardous Materials 163(2-3):625-631.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route,	Terrestrial; 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; Brassica rapa; var. chinensis; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Dibutyl phthalate (DBP)
HERO ID:	1296241

Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	High	DBP was identified by CAS number.	
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be Riedel-deHaen Co. in Germany, but it was not reported if it was analytically verified.	
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate solvent control.	
	Metric 5:	Negative Control Response	High	The negative control response was adequate and was reported in Table 3 in terms of biomass.	
	Metric 6:	Randomized Allocation	Low	It was not reported how the cabbage was allocated into study groups.	
Domain 3: Exposure Ch	aracterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	DBP was added to acetone at a concentration of 100g/L to create the stock solution. It was not reported how the test concentrations were prepared. It was also not reported how often new test solution was added to the system. The type of experimental vessel (plastic or glass) used is not reported.	
	Metric 8:	Consistency of Exposure Administration	Medium	All test exposures were conducted in 3L pots with 2.5L of solution at the same tempera- ture and relative humidity. Photoperiod was 16L:8D with 25C during the day and 20C at night. It was not reported how often the test solution was replaced.	
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP was measured at any point in the study.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of the study was reported to be 42d. This was adequate to observe a response.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Study authors reported 4 study groups plus a solvent control. This was adequate for the outcomes of interest.	
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of a vehicle solvent. The solvent control response was appropriate.	
Domain 4: Test Organis	m				
C	Metric 13:	Test Organism Characteristics	Low	The source of the cabbage seeds was not reported.	
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the plants were acclimated in any way to test conditions.	
	Metric 15:	Conditions Number of Organisms and	Low	The number of test organisms in each replicate was not reported. It was reported that	
		Replicates per Group		tests were carried out in triplicate.	
Continued on next page					

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Dibutyl Phthalate

		contin	ued from previ	ous page		
Study Citation: Duration: Exposure Route, Madia Path:	Liao, C. S., Journal of H Overall Dura Terrestrial; V	Liao, C. S., Yen, J. H., Wang, Y. S. (2009). Growth inhibition in Chinese cabbage (Brassica rapa var. chinensis) growth exposed to di-n-butyl phthalate. Journal of Hazardous Materials 163(2-3):625-631. Overall Duration: > 21 days; Exposure Duration: > 21 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	Vegetation:	Vascular Plants: Brassica rana: var. chinensi	s. Not Applicab	le (e.g. fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth	is, Not Applicab	ic (e.g., rungi of argae studies) of Not Reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1296241					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment					
	Metric 16:	Adequacy of Test Conditions	High	Authors reported growing the organisms in a solution adapted from Hoagland's solution. There was 2.5L of media in each pot. Organisms were grown at 25C during the day and 20C at night with a 16L:8D photoperiod. Relative humidity was reported to be 70-95%.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-development and growth of the plants in terms of biomass and in terms of physical changes in leaf color and etiolation.		
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the evaluation of of the plant development, morphology, and biomass were limited.		
Domain 6: Confoundin	a / 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. It was not reported if the plants 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 Present	tation and Anal	vsis				
	Metric 21:	Statistical Methods	High	T-tests were used to analyze data.		
	Metric 22:	Reporting of Data	High	Physiological differences were reported in Figure 2 for both treatment and control plants. Biomass for all exposure levels and for the control was reported in Table 3.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 3.		
Additional Comments:	This portion was taken. D	of the evaluation was on the effect of DBP or Development and growth was chosen as the o	n plant developm	nent and growth over 42 days. Leaf size and coloring were monitored, and biomass		

Overall Quality Determination

Medium

Study Citation:	Liao, C. S.,	Yen, J. H., Wang, Y. S. (2006). Effects of en	docrine disruptor	r di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis).		
Duration	Chemospher	Chemosphere $65(10)$:1715-1722. Overall Duration: > 21 days: Exposure Duration: > 21 days				
Exposure Route,	Terrestrial; V	Terrestrial; Water; Root uptake				
Media, Path:	,	· · ·				
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Brassica rapa</i> ; subsp. chind	ensis; Juvenile			
Health Outcome: Chemical:	Mechanistic Dibutyl phth	-Biomarkers (exposure and effect)-Oxidativ	e stress (includin	g redox biology)-Photosynthesis		
HERO ID:	1298079					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CAS number.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Riedel-deHaen Co. in Germany. The DBP was analytically verified during the residual DBP analysis part of the study, by GC/MS.		
	Metric 3:	Test Substance Purity	Hıgh	The purity was reported to be 98.7%.		
Domain 2: Test Design						
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control. However, they did not specify if a solvent control was used. Acetone was used as a vehicle solvent, so a solvent control would be necessary.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and was reported in Tables 2 and 3 for photosynthesis and in Figure 4 for the proteomics portion of the study.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the Bok choy was allocated into study groups.		
Domain 2: Exposure Ch	aractorization					
Domain 5. Exposure Ch	Metric 7.	Experimental System/Test Media	Low	Study authors reported dissolving DRP in acetone at a concentration of 100g/L but they		
	incure /.	Preparation	Low	did not elaborate on how the test concentrations were prepared. It was also not reported how often new test solution was added to the system.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 2.5L of test solution at the same temperature and rela- tive humidity. It was not reported how often the test solution was renewed, which creates doubt regarding the consistency.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the DBP was measured at any point in the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be 42 days and was appropriate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/	High	There were five concentrations plus a control. Spacing was adequate to see a response.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Study authors reported using a vehicle solvent, but they did not report if the negative control was a solvent control, so it cannot be determined if the solvent concentration was appropriate.		
Domain 4. Test Organis						
20mmin 1. rest Organis	Metric 13:	Test Organism Characteristics	Low	The source of the Bok choy seeds was not reported.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions.		
	Continued on next page					

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Environmental Hazard Evaluation

HERO ID: 1298079 Table: 1 of 3

		contin	ued from previ	ous page			
Study Citation:	Liao, C. S., Chemospher	Liao, C. S., Yen, J. H., Wang, Y. S. (2006). Effects of endocrine disruptor di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis). Chemosphere 65(10):1715-1722.					
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; V	Vater; Root uptake					
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica rapa; subsp. chine	nsis; Juvenile				
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Oxidative	stress (includir	ng redox biology)-Photosynthesis			
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1298079						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	It was reported that the tests were completed in triplicate, but the number of plants per			
		Replicates per Group		treatment was low: four plants each.			
Domain 5: Outcome A	ssessment						
Domain 5. Outcome A	Metric 16:	Adequacy of Test Conditions	High	Organisms were kept at 25C during the day and 20C at night with a 16L:8D photope-			
				riod. Relative humidity was at 70-95%. A modification of Hoagland's medium was used in the hydroponic growth chambers.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-chlorophyll concentration and chloroplast morphology and protein expression.			
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment were reported for chlorophyll concentration and			
		Assessment		chloroplast morphology in sections 5.2 and 5.1.			
Domain 6: Confoundir	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. It was not re- ported if the plants 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 attrition or health outcomes unrelated to exposure.			
Domain 7: Data Preser	tation and Anal	vsis					
Domain 7. Data 110501	Metric 21.	Statistical Methods	Low	Study authors did not report conducting statistical analysis, but independent analysis			
	Metric 21,	Statistical methods	Low	may be conducted from the data provided.			
	Metric 22:	Reporting of Data	High	Data for control response and exposure related findings was adequate and can be found in Figure 2 and Tables 2 and 3 for photosynthesis and in Figure 4 for the protein expres- sion.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.			

Additional Comments: This portion of the evaluation was on the effect of DBP on Bok choy chlorophyll concentration and chloroplast morphology as well protein expression.

Overall Quality Determination

Medium

Study Citation: Duration:	Liao, C. S., Yen, J. H., Wang, Y. S. (2006). Effects of endocrine disruptor di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis). Chemosphere 65(10):1715-1722. Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Terrestrial; V Vegetation; V Developmen Dibutyl phth 1298079	Terrestrial; Water; Root uptake Vegetation; Vascular Plants; <i>Brassica rapa</i> ; subsp. chinensis; Juvenile Development/Growth Dibutyl phthalate (DBP) 1298079				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	The DBP was identified by CAS number.		
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Riedel-deHaen Co. in Germany. The DBP was analytically verified during the residual DBP analysis part of the study, by GC/MS.		
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%		
Domain 2: Test Design			_			
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control. However, they did not specify if a solvent control was used. Acetone was used as a vehicle solvent, so a solvent control would be necessary.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and was reported in Table 3 and Figure 1.		
	Metric 6:	Randomized Allocation	Low	It was not reported how the Bok choy was allocated into study groups.		
	, · ,·					
Domain 3: Exposure Ch	Aracterization	E/T/T/T/T	T			
	Metric 7:	Preparation	Low	did not elaborate on how the test concentrations were prepared. It was also not reported how often new test solution was added to the system.		
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 2.5L of test solution at the same temperature and rela- tive humidity. It was not reported how often the test solution was renewed, which creates doubt regarding the consistency.		
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the DBP was measured at any point in the study.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be 42 days and was appropriate for the outcomes of interest.		
	Metric 11:	Number of Exposure Groups/	High	There were 5 concentrations plus a control. Spacing was adequate to see a response.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Study authors reported using a vehicle solvent, but they did not report if the negative control was a solvent control, so it cannot be determined if the solvent concentration was appropriate.		
Domain 4: Test Organis	m					
Domain 4. Test Organisi	Metric 13:	Test Organism Characteristics	Low	The source of the Bok chov seeds was not reported.		
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions.		
		Conti	nued on next pa	ge		

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		contin	ued from previ	ous page			
Study Citation:	Liao, C. S., Y	Liao, C. S., Yen, J. H., Wang, Y. S. (2006). Effects of endocrine disruptor di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis).					
Duration:	Overall Dura	Overall Duration: > 21 days: Exposure Duration: > 21 days					
Exposure Route.	Terrestrial: V	Terrestrial: Water: Root untake					
Media. Path:	, ,						
Taxa, Species, Age:	Vegetation: V	Vascular Plants: <i>Brassica rana</i> : subsp. chine	nsis: Juvenile				
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1298079						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	It was reported that the tests were completed in triplicate, but the number of plants per			
		Replicates per Group		treatment was low: four plants each.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Organisms were kept at 25C during the day and 20C at night with a 16L:8D photope- riod. Relative humidity was at 70-95%. A modification of Hoagland's medium as used in the hydroponic growth chambers.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-development and growth of the Bok choy.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details of the outcome assessment were limited for biomass determination and for plant morphology.			
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. It was not re- norted if the plants 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 attrition or health outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	vsis					
2 shun /. Duu 1 resen	Metric 21:	Statistical Methods	Low	Study authors did not report conducting statistical analysis, but independent analysis may be conducted from the data provided.			
	Metric 22:	Reporting of Data	High	Data for control response and exposure related findings was adequate and can be found in Figure 1 and Table 3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the table.			
Additional Comments:	This portion outcome of i	of the evaluation was on the effect of DBP nterest.	on Bok choy b	omass and plant morphology/coloring. Development/growth was selected as the			

Overall Quality Determination

Medium

Study Citation: Duration:	Liao, C. S., Yen, J. H., Wang, Y. S. (2006). Effects of endocrine disruptor di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis). Chemosphere 65(10):1715-1722. Overall Duration: > 21 days; Exposure Duration: > 21 days				
Exposure Route,	Terrestrial; V	Vater; Root uptake			
Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V ADME (biot Dibutyl phth 1298079	Vascular Plants; <i>Brassica rapa</i> ; subsp. chine ransformation) alate (DBP)	ensis; Juvenile		
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CAS number.	
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be Riedel-deHaen Co. in Germany. The DBP was analytically verified during the residual DBP analysis part of the study, by GC/MS.	
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.7%	
Domain 2: Test Design					
	Metric 4:	Negative Controls	Low	Study authors reported the use of a concurrent negative control. However, they did not specify if a solvent control was used. Acetone was used as a vehicle solvent, so a solvent control would be necessary.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control was adequate and was reported in Tables 1 and 3.	
	Metric 6:	Randomized Allocation	Low	It was not reported how the Bok choy was allocated into study groups.	
Domain 2: Exposure Ch	aractorization				
Domain 5: Exposure Ch	Motrio 7:	Experimental System/Test Media	Low	Study outhors reported discoluting DRD in contains at a concentration of 100 c/L but they	
	Meure 7.	Preparation	Low	did not elaborate on how the test concentrations were prepared. It was also not reported how often new test solution was added to the system.	
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were conducted in 2.5L of test solution at the same temperature and rela- tive humidity. It was not reported how often the test solution was renewed, which creates doubt regarding the consistency.	
	Metric 9:	Measurement of Test Substance	Low	Study authors did not report if the DBP was measured at any point in the study.	
	Metric 10:	Concentration Exposure Duration and Frequency	High	The study duration was reported to be 42 days and was appropriate for the outcomes of interest.	
	Metric 11:	Number of Exposure Groups/	High	There were 5 concentrations plus a control. Spacing was adequate to see a response.	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Medium	Study authors reported using a vehicle solvent, but they did not report if the negative control was a solvent control, so it cannot be determined if the solvent concentration was appropriate.	
Domain 4. Toot Ore					
Domain 4: Test Organisi	m Metric 13.	Test Organism Characteristics	Low	The source of the Bok choy was not reported	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions.	
		Conti	nued on next pa	ge	

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		contin	ued from previ	ous page			
Study Citation:	Liao, C. S., Chemospher	Yen, J. H., Wang, Y. S. (2006). Effects of end re 65(10):1715-1722	locrine disrupto	r di-n-butyl phthalate on the growth of Bok choy (Brassica rapa subsp. chinensis).			
Duration:	Overall Dura	Overall Duration: >21 days: Exposure Duration: >21 days					
Exposure Route,	Terrestrial; V	Terrestrial: Water: Root uptake					
Media, Path:	,						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica rapa; subsp. chiner	nsis; Juvenile				
Health Outcome:	ADME (bio	transformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1298079						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and	Low	It was reported that the tests were completed in triplicate, but the number of plants per			
		Replicates per Group		treatment was low: four plants each.			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	High	Organisms were kept at 25C during the day and 20C at night with a 16L:8D photope- riod. Relative humidity was at 70-95%. A modification of Hoagland's medium as used in the hydroponic growth chambers.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DBP accumulation in Bok choy tissues.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Assessment was described in detail in section 2.4.			
Domain & Confoundin	a / Variabla Ca	ntanl					
Domain 6: Comoundin	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	metale 17.	Design and Procedures	2011	conditions or other non-treatment-related factors across study groups. It was not re- ported if the plants 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 attrition or health outcomes unrelated to exposure.			
Domain 7: Data Presen	tation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Study authors did not report conducting statistical analysis, but independent analysis may be conducted from the data provided.			
	Metric 22:	Reporting of Data	High	Data for control response and exposure related findings was adequate and can be found in Figure 3 and in Tables 1 and 3.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.			

Overall Quality Determination

Medium

Study Citation:	Zhu, F., Zhu	Zhu, F., Zhu, C., Chen, N., Zhou, D., Gao, J. (2018). Will spent mushroom substrate application affect the dissipation and plant uptake of phthalate esters?.				
Duration: Exposure Route.	Journal of So Overall Dura Terrestrial: S	Journal of Soils and Sediments 18(4):1579-1589. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial: Soil: Root uptake				
Media, Path:	Terrestriar, e	in, noor uptake				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Brassica rapa; Subspecies: 0	Chinensis; Embryo			
Health Outcome:	ADME (biot	ransformation)				
HERO ID:	5605728					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	High	CAS number, nomenclature and structure were reported by the authors.		
	Metric 2:	Test Substance Source	Low	Source was reported (Sigma) but not verified.		
	Metric 3:	Test Substance Purity	High	The purity from the manufacturer was reported.		
Domain 2. Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	Low	Control soil was reported but authors did not detail if it was a solvent or abiotic control.		
	Metric 5:	Negative Control Response	Uninformative	The control soil without DPB added at 50mg/kg has several phthalates in it already indicating the soil is contaminated. As a result, the ability to determine ADME between control and treated samples is not possible.		
	Metric 6:	Randomized Allocation	Medium	Allocation was reported as random.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media	Medium	The addition of DBP in the soil was well described and appeared to be appropriate.		
	Metric 8:	Consistency of Exposure	High	There was only one exposure group at 50mg/kg in the soil.		
	Metric 9:	Administration Measurement of Test Substance	Low	Authors did not verify treatment soil concentrations.		
	Metric 10:	Concentration Exposure Duration and Frequency	High	The authors allowed for 30 days of seedling growth with a previous 7-day germination period.		
	Metric 11:	Number of Exposure Groups/	N/A	The authors only had one concentration of DBP (50 mg/kg).		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via soil.		
Domain 4. Test Organis	m					
Zomun I. Test Organis	Metric 13:	Test Organism Characteristics	Medium	The source of seeds was listed, but the storage conditions of seeds prior to the experi- ment were not described.		
	Metric 14:	Acclimatization and Pretreatment	Medium	Conditions before germination were not described.		
	Metric 15:	Conditions Number of Organisms and	Low	Authors reported six seeds per pot, but no record of the number of pots per treatment		
		Replicates per Group		group or representation. Samples were represented for analytical analysis.		

Domain 5: Outcome Assessment

Continued on next page ...

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Dibutyl Phthalate

		com	tinued from previous	page			
Study Citation: Duration: Exposure Route, Media, Path:	Zhu, F., Zhu, Journal of So Overall Dura Terrestrial; S	Zhu, F., Zhu, C., Chen, N., Zhou, D., Gao, J. (2018). Will spent mushroom substrate application affect the dissipation and plant uptake of phthalate esters?. Journal of Soils and Sediments 18(4):1579-1589. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake					
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Brassica rapa; Subspecies: Cl	hinensis; Embryo				
Health Outcome:	ADME (biot	ransformation)					
HERO ID:	5605728						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	Soil conditions were described and environmental conditions were controlled.			
	Metric 17:	Outcome Assessment Methodology	Low	Table 5 reports the DBP concentrations, however, all plants have other phthalates that indicate controlled conditions for the uptake of just DBP were not possible.			
	Metric 18:	Consistency of Outcome Assessment	High	Assessment appears to be consistent among treatment and control.			
Domain 6: Confounding	g / Variable Cor	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors do not report any differences among groups that would influence results.			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing provided indicates that differences were due to plant health or attrition.			
Domain 7: Data Present	tation and Anal	ysis					
	Metric 21:	Statistical Methods	High	Authors used ANOVA with Tukey post-hoc to determine differences among treatment groups.			
	Metric 22:	Reporting of Data	Low	Values are presented for DBP concentrations as Means +/- SD but some parts of the plant are only presented as single values.			
	Metric 23:	Explanation of Unexpected Outcomes	Medium	It is not clear why other phthalate concentrations are present in Table 5, if control soil was not initially contaminated.			
Additional Comments:	There is phth	nalate contamination in the control soil. Expos	ure concentrations were	e not verified.			

Overall Quality Determination

Uninformative

Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-				
Duration:	163. Overall Dura	ation: Not-reported; Exposure Duration: N	lot-reported			
Exposure Route,	Terrestrial; A	Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Media, Path: Taxa Spacios Ago:	Vagatation					
Health Outcome:	Mechanistic	-Photosynthesis	r, Not Applicable (e.g., lungi	of algae studies) of Not Reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1333234					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		т			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 3:	Test Substance Purity	Medium	Pro analysis quality was reported		
	intenite or					
Domain 2: Test Design			_			
	Metric 4:	Negative Controls	Low	Untreated plants were used as controls but few details were provided.		
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.		
	Metric 0.	Randomized Anocation	LOW	Researchers did not report now organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
	Metric 7:	Experimental System/Test Media Preparation	Medium	Several methods of obtaining vapors were reported but effects were not attributed to a particular method.		
	Metric 8:	Consistency of Exposure	Low	It was difficult to determine as durations and concentrations were not clearly reported.		
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported.		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days, but study length was not clearly reported.		
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the number of exposure groups, the range seems to be		
		Spacing of Exposure Levels		10-150 ng/L, but it's unclear if this is one treatment that varied over time or if there were multiple treatment levels.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via vapors.		
Domain 1. Test Organia	m					
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, and age or stage at test initiation was not		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.		
	16.1.1.6	Conditions	Ŧ			
	Metric 15:	Number of Organisms and	Low	Numbers and replicates were not reported.		
		Replicates per Group				
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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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333234 Table: 1 of 3

		con	tinued from previou	s page		
Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-				
Duration: Exposure Route, Media, Path: Taxa, Species, Age:	163. Overall Dura Terrestrial; A Vegetation; V	 163. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Browallia speciosa</i>; Major; Not Applicable (e.g., fungi or algae studies) or Not Reported 				
Health Outcome:	Mechanistic	-Photosynthesis				
HERO ID:	Dibutyl phthalate (DBP) 1333234					
Domain		Metric	Rating	Comments		
	Metric 18: Consistency of Outcome Assessment		Low	Details regarding the execution of the study protocol for outcome assessment were con- fusing.		
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.		
Domain 7: Data Preser	ntation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, and information was not quantified.		
	Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.		
Additional Comments:	This was a c	losed system and light was a variable.				

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1333234 Table: 2 of 3

Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-			
Duration	163. Overall Dura	163. Overall Duration: Not-reported: Exposure Duration: Not-reported			
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e., c	chemical of interest in expo	sure water, but unable to determine exact uptake route)	
Media. Path:					
Taxa. Species. Age:	Vegetation: Vascular Plants; Browallia speciosa; Major: Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mortality				
Chemical:	Dibutyl phthalate (DBP)				
HERO ID:	1333234				
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	High	The test substance identity was analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	Medium	pro analysis quality was reported	
Domain 2: Test Design					
	Metric 4:	Negative Controls	Low	Untreated plants were used as controls but few details were provided	
	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	Medium	Several methods of obtaining vapors was reported but effects were not attributed to a particular method	
	Metric 8:	Consistency of Exposure	Low	Difficult to determine as durations and concentrations were not clearly reported	
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported	
	Metric 10:	Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days but study length was not clearly reported	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups, the range seems to be 10-150 ng/L but unclear if this is one treatment that varied over time or if there were multiple treatment levels	
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via vapors	
Domain 4. Test Organis	m				
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, age or stage at test initiation was not clear.	
	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	Numbers and replicates were 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	
	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 .		

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333234 Table: 2 of 3

		c	ontinued from previous p	age		
Study Citation:	Virgin, H. I.,	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-				
Duration:	163. Overall Dura	163. Overall Duration: Not-reported; Exposure Duration: Not-reported				
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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 Mortality Dibutyl phth 1333234	Vegetation; Vascular Plants; <i>Browallia speciosa</i> ; Major; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality Dibutyl phthalate (DBP) 1333234				
Domain	Metric Rating Comments					
Domain 6: Confounding	g / Variable Con Metric 19:	ntrol 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		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, information was not quantified		
	Metric 22:	Reporting of Data	Low	There was brief mention of plant mortality in the results section text. No data was shown.		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability		
Additional Comments:	closed syster	m, light was a variable				
Overall Quali	ty Detern	nination	Uninformative			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-					
Duration:	163. Overall Dura	163. Overall Duration: Not-reported: Exposure Duration: Not-reported				
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; Browallia speciosa; Major;	Not Applicable (e.g., fungi	or algae studies) or Not Reported		
Health Outcome:	Mechanistic-	Cytotoxicity-Photosynthesis				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1333234					
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	High	The test substance identity was analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Medium	pro analysis quality was reported		
Domain 2: Test Design						
6	Metric 4:	Negative Controls	Low	Untreated plants were used as controls but few details were provided		
	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	Medium	Several methods of obtaining vapors was reported but effects were not attributed to a		
		Preparation		particular method		
	Metric 8:	Consistency of Exposure	Low	Difficult to determine as durations and concentrations were not clearly reported		
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days but study length was not clearly reported		
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the number of exposure groups, the range seems to be		
		Spacing of Exposure Levels		10-150 ng/L but unclear if this is one treatment that varied over time or if there were multiple treatment levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via vapors		
Domain 4: Test Organis	m Matria 12	Test Organism Characteristics	Low			
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, age or stage at test initiation was not clear.		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized		
	Metric 15:	Conditions Number of Organisms and	Low	Numbers and replicates were 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 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 con-		
		Assessment		fusing		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 1333234 Table: 3 of 3

		co	ntinued from previous	page	
Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-			
Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	163. Overall Dura Terrestrial; A Vegetation; V Mechanistic Dibutyl phth 1333234	 163. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vegetation; Vascular Plants; <i>Browallia speciosa</i>; Major; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Cytotoxicity-Photosynthesis Dibutyl phthalate (DBP) 1333234 			
Domain		Metric	Rating	Comments	
Domain 6: Confounding	g / Variable Con 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	
Domain 7: Data Present	tation and Anal	lysis			
	Metric 21: Metric 22: Metric 23:	Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	N/A Low Low	Statistical analysis was not possible, information was not quantified Data were only reported for some outcomes The study did not report any measures of variability	
Additional Comments:	open system	, light was a variable			
Overall Quali	ty Detern	nination	Uninformative	e	

Dibutyl Phthalate

Study Citation:	Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis					
D (1	recovery. Jo	covery. Journal of Plant Nutrition 10(9-16):1051-1058.				
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: Not	-reported	(nocure water, but unable to determine exact untel/e route)		
Exposure Route, Modia Path:	Terresultar,	water, Not determined by study authors (i.e.,	chemical of interest in ex	(posure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation	Vegetation; Vascular Plants; Carica papaya; Co-1; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Mechanistic	Mechanistic-Cell signaling/function-Nutritional and Metabolic				
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	5433168					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ice		-			
	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						
C	Metric 4:	Negative Controls	Low	Author reported a treatment without DBP or caffeic acid		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcome		
	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 no details on the measures taken to appropriately prepare test con-		
	Matria 8.	Preparation Consistency of Exposure	Low	centrations		
	Metric 8.	Administration	Low	No details of exposure administration were reported		
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured		
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	duration was not reported		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration tested		
		Spacing of Exposure Levels				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit		
Domain 1. Test Organis	m					
Domain 4. 10st Organis	Metric 13.	Test Organism Characteristics	Low	The source of the test plants was not reported		
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized		
		Conditions	20.0	The study and net report whether rest organisms were detriminanted		
	Metric 15:	Number of Organisms and	Low	The number of test organisms and/or replicates was not reported.		
		Replicates per Group				
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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Dibutyl Phthalate

HERO ID: 5433168 Table: 1 of 3

		CO	ntinued from previous	page		
Study Citation:	Kannan, S.,	Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis				
	recovery. Jou	recovery. Journal of Plant Nutrition 10(9-16):1051-1058.				
Duration:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported				
Exposure Route,	Terrestrial; V	Water; Not determined by study authors (i.e., o	chemical of interest in ex	(posure water, but unable to determine exact uptake route)		
Media, Path:	N7	Versien Diseter Casis a server Co. 1. Not As		alaan atadiaa) ah Mat Damartad		
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Carica papaya</i> ; Co-1; Not Aj	pplicable (e.g., lung) or a	argae studies) or Not Reported		
Health Outcome:	Dibutul abth	-Cell signaling/lunction-inutritional and Meta	idone			
Unemical:	5/22169	alate (DDP)				
	3433108					
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	ntrol	Ŧ			
	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		
Domain 7: Data Present	ation and Anal	veie				
Domani 7. Data Present	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 22:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability		
	metric 23.	Explanation of Onexpected Outcomes	LOW	The study did not report any measures of variability		
Additional Comments:	None					
Overall Quali	ty Detern	nination	Uninformative	2		

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HERO ID: 5433168 Table: 2 of 3

Study Citation:	Kannan, S.,	Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis					
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; V	ecovery. Journal of Plant Nutrition 10(9-16):1051-1058. Overall Duration: Not-reported; Exposure Duration: Not-reported Ferrestrial; 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	egetation; Vascular Plants; <i>Carica papaya</i> ; Co-3; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-	echanistic-Cell signaling/function-Nutritional and Metabolic					
HERO ID:	5433168	ibutyl phthalate (DBP) 133168					
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							
C	Metric 4:	Negative Controls	Low	Author reported a treatment without DBP or caffeic acid			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcome			
	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 no details on the measures taken to appropriately prepare test con- centrations			
	Metric 8:	Consistency of Exposure	Low	No details of exposure administration were reported			
		Administration					
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	duration was not reported			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration tested			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 1. Test Organics	m						
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of the test plants 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 and/or 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 adequate			
	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			
				-			

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Environmental Hazard Evaluation

HERO ID: 5433168 Table: 2 of 3

			ntinued from previous	page	
Study Citation:	Kannan, S.,	Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis			
	recovery. Jo	urnal of Plant Nutrition 10(9-16):1051-1058.			
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: Not-	reported		
Exposure Route,	Terrestrial; V	Water; Not determined by study authors (i.e., o	chemical of interest in ex	sposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Carica papaya; Co-3; Not Aj	pplicable (e.g., fungi or a	algae studies) or Not Reported	
Health Outcome:	Mechanistic	-Cell signaling/function-Nutritional and Meta	bolic		
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)			
HERO ID:	5433168				
Domain		Metric	Rating	Comments	
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	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	Low	The study did not report any measures of variability	
Additional Comments:	None				

Dibutyl Phthalate

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HERO ID: 5433168 Table: 3 of 3

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	 Kannan, S., Ramani, S. (1987). Mechanisms of fe-deficiency tolerance in crop cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis recovery. Journal of Plant Nutrition 10(9-16):1051-1058. Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial; 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>Carica papaya</i>; Co-4; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Cell signaling/function-Nutritional and Metabolic Dibutyl phthalate (DBP) 5433168 				
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	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design					
C	Metric 4:	Negative Controls	Low	Author reported a treatment without DBP or caffeic acid.	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for the assessed outcome.	
	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 no details on the measures taken to appropriately prepare test con- centrations.	
	Metric 8:	Consistency of Exposure	Low	No details of exposure administration were reported.	
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.	
	Metric 10:	Concentration Exposure Duration and Frequency	Uninformative	Exposure duration was not reported.	
	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	High	Exposure concentrations were below the water solubility limit.	
Domain 4: Test Organis	m		-		
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates were not reported.	
		E			
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.	
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.	

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 5433168 Table: 3 of 3

		col	ntinued from previous	page
Study Citation:	Kannan, S.,	Ramani, S. (1987). Mechanisms of fe-defic	eiency tolerance in crop	cultivars - effects of dibutyl phthalate and caffeic acid on fe-chlorosis
	recovery. Jo	urnal of Plant Nutrition 10(9-16):1051-1058.		
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: Not-	reported	
Exposure Route,	Terrestrial; V	Water; Not determined by study authors (i.e., o	chemical of interest in ex	posure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Carica papaya; Co-4; Not Ap	pplicable (e.g., fungi or a	algae studies) or Not Reported
Health Outcome:	Mechanistic	-Cell signaling/function-Nutritional and Meta	bolic	
Chemical:	Dibutyl phth	alate (DBP)		
HERO ID:	5433168			
Domain		Metric	Rating	Comments
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	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	Low	The study did not report any measures of variability.
Additional Comments:	None			

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Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth							
Duration	regulators. S	egulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topical application)							
Media, Path:								
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; cv. National Pickling stock; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Development/Growth Dibutyl phthalate (DBP)							
Chemical: HFRO ID:	Dibutyl phth	alate (DBP)						
	5551990							
Domain Domain 1: Tast Substan	22	Metric	Rating	Comments				
Domain 1. Test Substan	Metric 1.	Test Substance Identity	High	Chemical name and structure were used to identify the test chemical				
	Metric 2:	Test Substance Source	Low	The source was not reported.				
	Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent solvent control group.				
	Metric 5:	Negative Control Response	Low	The biological response of the solvent control group was not reported.				
	Metric 6:	Randomized Allocation	Low	A selection criteria was indicated (selected seedlings with 3 cm of hypocotyl), but de- tails regarding assignment to groups were 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 test concentrations were not measured. Vessel material was not reported.				
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.				
	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 suitable for the study type.				
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations were used, but a wide range was tested.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Both treatment solutions and the control solution were prepared with 90% ethanol, which is miscible with DBP. The biological response of the solvent control was not reported.				
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	High	Cucumber are an appropriate species for this assay and the source of the seeds was reported.				
	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 and replicates was not reported.				

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Environmental Hazard Evaluation

HERO ID: 5551990 Table: 1 of 1

		co	ntinued from previous	page			
Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Duration:							
Exposure Route,	Terrestrial; N/A (e.g., injection); Dermal (topical application)						
Media, Path:							
Taxa, Species, Age:	Vegetation; Vascular Plants; Cucumis sativus; cv. National Pickling stock; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	5551990						
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 adequate.			
	Metric 17:	Outcome Assessment Methodology	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 lim-			
		Assessment		ited.			
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.			
Domain 7: Data Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted and no data was provided.			
	Metric 22:	Reporting of Data	Low	Data were not shown for treatment or control groups, but results were described in the			
				text.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion of the study was on the plant growth promoting activity of DBP on Cucumis sativus cv National Pickling stock. Development/Growth was selected as the outcome of interest. This portion of the study is rated unacceptable as statistical analysis was not conducted and no numerical data was provided.						

Dibutyl Phthalate

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, Media, Path:	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: <i>Cucumis sativus</i> : Embryo					
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis					
Chemical:	Dibutyl phthalate (DBP)					
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	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 adequate		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups		
Domain 3: Exposure Characterization						
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	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 Organism						
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	Medium	The numbers of test organisms and replicates were reported and sufficient to character-		
		Replicates per Group		ize toxicological effects		
Domain 5: Outcome Assessment						
	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, 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	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; Cucumis sativus; Embryo			
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis		
Chemical:	Dibutyl phth	nalate (DBP)			
HERO ID:	2915866				
Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups	
		Assessment	U		
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	
	Medie 19.	Design and Procedures	mgn	There were no reported unreferees 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	tation 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:	Pigment con	tent"The results indicate that longer period	ls of cultivati	ion of the test plants may make it easier to interpret the changes in pigment contents."	
Overall Quali	ty Deterr	nination	High		

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Ma, T., Teng Frontiers of D Overall Dura Terrestrial; S Vegetation; V	g, Y., Christie, P., Luo, Y. (2015). Phytoto Environmental Science & Engineering 9(2 ation: 0 - 4 days (0-96h); Exposure Duratic Goil; Not determined by study authors (i.e., Vascular Plants; <i>Cucumis sativus</i> ; Embryo	xicity in seve):259-268. on: 0 - 4 days chemical of	n higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. (0-96h) interest in exposure water, but unable to determine exact uptake route)
Health Outcome: Chemical: HERO ID:	Reproductive Dibutyl phth 2915866	e/Teratogenic alate (DBP)		
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				
	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 en	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/ 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.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

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Dibutyl Phthalate

continued from previous page						
Study Citation:	Ma, T., Teng Frontiers of I	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	tion: 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., o	chemical of i	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation; V	/ascular Plants; Cucumis sativus; Embryo				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	/ Variable Con	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	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.		
Domain 7: Data Presenta	tion and Analy	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:	This form rep	The were no unexpected outcomes and the were no unexpected outcomes.				

Overall Quality Determination

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>Cucumis sativus</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 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						
2 Sinum 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	ing.	response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds were 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 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	High High	The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups.		
		Assessment				

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Dibutyl Phthalate

continued from previous page							
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.					
	Frontiers of	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; Y	Vascular Plants; Cucumis sativus; Embryo					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
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 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 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 Qualit	y Deterr	nination	High				

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age:	Wang, L., Sun, X., Chang, Q., Tao, Y., Wang, L., Dong, J., Lin, Y., Zhang, Y. (2016). Effect of di-n-butyl phthalate (DBP) on the fruit quality of cucumber and the health risk. Environmental Science and Pollution Research 23(23):24298-24304. Overall Duration: Not-reported; Exposure Duration: Not-reported 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> : Invenile							
Health Outcome:	Mechanistic	Mechanistic-Reproductive/Teratogenic						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	3502464							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ice							
	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.				
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control with black soil only.				
	Metric 5:	Negative Control Response	High	The response of the negative control was reported in Table 1 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Medium	It was reported that a random block design was used for this study.				
Domain 3: Exposure Ch	naracterization	Experimental System/Test Media	Medium	The test media was prepared by making a stock solution of 100mg/mL DBP in ethanol				
	Wette 7.	Preparation	Wedium	and then adding that in the appropriate amount to black soil. The ethanol was allowed to evaporate before the soil was used for the study. The tests took place in $40 \times 80 \times 40$ class boxes.				
	Metric 8:	Consistency of Exposure Administration	Medium	All tests took place in class boxes of the same size with one seedling each until the harvest of the fruit.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP concentrations were measured at any point in the study.				
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The study was reported to be from the time of a seedling with three leaves until fruit harvest. This was given a low because the fruit was the central point of interest in the study, so harvest was an appropriate stopping point, but study authors did not report the number of days from the start of the study to the harvest.				
	Metric 11:	Number of Exposure Groups/	High	There were four exposure levels, and the spacing was appropriate for a response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.				
Domain 4: Test Organis	m							
Domain 4. Test Organis	Metric 13.	Test Organism Characteristics	Low	The source of the cucumber seeds was not reported				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.				
	Metric 15:	Conditions Number of Organisms and	Medium	There was one organism per test chamber and five replicates per test concentration.				
		Replicates per Group						
		Contin	nued on next pa	ge				

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HERO ID: 3502464 Table: 1 of 2

		contin	ued from previ	ous page			
Study Citation:	Wang, L., Su	Wang, L., Sun, X., Chang, Q., Tao, Y., Wang, L., Dong, J., Lin, Y., Zhang, Y. (2016). Effect of di-n-butyl phthalate (DBP) on the fruit quality of cucumber and the health risk. Environmental Science and Pollution Research 23(23):24298-24304					
Duration:	Overall Dura	Overall Duration: Not-reported; Exposure Duration: Not-reported					
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:	Vegetation; Vascular Plants; Cucumis sativus; Juvenile						
Health Outcome:	Mechanistic-Reproductive/Teratogenic						
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	3502464						
Domain		Metric	Rating	Comments			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	It was reported that the cucumbers were kept at 27C during the day and 20C at night with a relative humidity of 70%. Watering was not reported nor was photoperiod.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–organic acid content, soluble protein content, soluble sugar content, and vitamin C content in cucumber fruits.			
	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. Contounding	Metric 19	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
	metile 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 Proconte	ation and Anal	lucio					
Domain 7. Data i lesella	Metric 21.	Statistical Methods	High	Statistical methods were described in the "statistical analyses" section of the paper			
	Metric 21: Metric 22:	Reporting of Data	High	The exposure response and the control response are presented in Table 1 and are ade- quate for the outcomes of interest.			
	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 of the evaluation was on the effect of cucumber fruit quality after exposure to various levels of DBP. Since fruit is the reproductive organ of the plant and the study authors were observing effects on organic acid, soluble protein, soluble sugar, and vitamin C content of the fruit, the mechanistic reproduction outcome was chosen.						

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Wang, L., Su and the healt Overall Dura Terrestrial; S Vegetation; V ADME (biot Dibutyl phth 3502464	un, X., Chang, Q., Tao, Y., Wang, L., Dong, h risk. Environmental Science and Pollution tion: Not-reported; Exposure Duration: No oil; Not determined by study authors (i.e., c Vascular Plants; <i>Cucumis sativus</i> ; Juvenile ransformation) alate (DBP)	J., Lin, Y., Zhan n Research 23(2: t-reported themical of inter-	g, Y. (2016). Effect of di-n-butyl phthalate (DBP) on the fruit quality of cucumber 3):24298-24304. est in exposure water, but unable to determine exact uptake route)
Domain		Matric	Dating	Comments
Domain 1: Test Substan	ce	Methe	Katilig	Comments
Domain 1. Test Substan	Metric 1:	Test Substance Identity	Low	The DBP was identified by name only.
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control with black soil only.
	Metric 5:	Negative Control Response	High	The response of the negative control was reported in Table 2 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Medium	It was reported that a random block design was used for this study.
Domain 3: Exposure Ch	Metric 7: Metric 8:	Experimental System/Test Media Preparation Consistency of Exposure Administration	Medium Medium	The test media was prepared by making a stock solution of 100mg/mL DBP in ethanol and then adding that in the appropriate amount to black soil. The ethanol was allowed to evaporate before the soil was used for the study. The tests took place in $40 \times 80 \times 40$ class boxes. All tests took place in class boxes of the same size with one seedling each until the harvest of the fruit.
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the DBP concentrations were measured at any point in the study.
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The study was reported to be from the time of a seedling with 3 leaves until fruit harvest. This was given a low because the fruit was the central point of interest in the study, so harvest was an appropriate stopping point, but study authors did not report the number of days from the start of the study to the harvest.
	Metric 11:	Number of Exposure Groups/	High	There were 4 exposure levels, and the spacing was appropriate for a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.
Domain 4: Test Organis	m			
8	Metric 13:	Test Organism Characteristics	Low	The source of the cucumber seeds was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if any acclimation occurred.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There was one organism per test chamber and 5 replicates per test concentration.
Domain 5: Outcome Ass	sessment	· ·		

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Dibutyl Phthalate

			-				
Study Citation:	Wang, L., Su and the healt	Wang, L., Sun, X., Chang, Q., Tao, Y., Wang, L., Dong, J., Lin, Y., Zhang, Y. (2016). Effect of di-n-butyl phthalate (DBP) on the fruit quality of cucumber and the health risk. Environmental Science and Pollution Research 23(23):24298-24304.					
Duration:	Overall Dura	ation: Not-reported; Exposure Duration: Not	-reported	, ,			
Exposure Route.	Terrestrial: S	Soil. Not determined by study authors (i.e. c)	hemical of intere	est in exposure water, but unable to determine exact untake route)			
Media Path.	ienestiai, c	in the determined by study dumons (ne., e					
Tomo Smootha A and	N/	Vagetation: Vagetaler Diante: Cucumic softwar Iswanila					
Taxa, Species, Age:	vegetation;	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Juvenile					
Health Outcome:	ADME (biot	transformation)					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	3502464						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	It was reported that the cucumbers were kept at 27C during the day and 20C at night with a relative humidity of 70%. Watering was not reported nor was photoperiod.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-accumulation of DBP in cucumber fruit tissue.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups–GC-MS was used to determine DBP levels in cucumber fruits.			
Domain 6: Confounding	g / Variable Co	ntrol					
2 children of Controlling	Metric 19	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.			
	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 in the "statistical analyses" section of the paper.			
	Metric 22:	Reporting of Data	High	The exposure response and the control response are presented in Table 2 and are ade- quate for the outcomes of interest.			
	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 of the evaluation was on the accumulation of DBP in cucumber fruit tissue. ADME was selected as the outcome of interest.

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route,	 Wu, Y., Yuan, S. L. (2012). Dibutyl phthalate pollution on cotton growth and physiological characteristics of cotton. Advanced Materials Research 518-523:5436-5441. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; 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:	Vegetation; V Reproductive Dibutyl phth 1639289	Vegetation; Vascular Plants; <i>Gossypium</i> ; Species not reported in text.; Embryo Reproductive/Teratogenic Dibutyl phthalate (DBP) 1639289					
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 DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	High	The 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 response and the solvent control response were reported in Figure 1 and in Table 1.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the cotton seeds were allocated into study groups.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the test system and on the preparation of the test con- centrations. Exposure was conducted in glass Petri dishes.			
	Metric 8:	Consistency of Exposure	Low	Little details were provided on the exposure administration.			
	Metric 9:	Administration Measurement of Test Substance	Low	It was not reported if the test concentrations were measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the germination and root length portion of the study was reported to be five days. This was adequate to observe a response.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 10 exposure levels plus a negative control and a solvent control. Spacing appeared adequate to observe a response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of acetone as a vehicle solvent. The solvent control re- sponse was adequate.			
Domain 4: Test Organisi	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the cotton seeds was not reported. The scientific name was also not re- ported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of cotton seeds used and the number of replicates was not reported.			

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Environmental Hazard Evaluation

HERO ID: 1639289 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation:	Wu, Y., Yua	Wu, Y., Yuan, S. L. (2012). Dibutyl phthalate pollution on cotton growth and physiological characteristics of cotton. Advanced Materials Research 518-					
Duration:	Overall Dura	523:5436-5441. Overall Duration: 4 - 10 days: Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; V	Water; Not determined by study authors (i.e	., chemical o	of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:	,		,				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Gossypium; Species not re	ported in text	t.; Embryo			
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1639289						
Domain		Metric	Rating	Comments			
Domain 5: Outcome A	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	The environmental conditions were not sufficiently reported to evaluate if they were adequate for organism health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-seed germination/reproduction.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol were not reported.			
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.			
	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	Low	Statistical analysis was performed but the methods used were not described.			
	Metric 22:	Reporting of Data	High	The control response and the exposure response for germination were reported in Figure 1 and in Table 1.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.			

Overall Quality Determination

Low

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HERO ID: 1639289 Table: 2 of 2

Study Citation:	Wu, Y., Yua	n, S. L. (2012). Dibutyl phthalate pollutio	n on cotton g	rowth and physiological characteristics of cotton. Advanced Materials Research 518-			
Duration: Exposure Route, Media Path	523:5436-54 Overall Dura Terrestrial; V	523:5436-5441. Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h) 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:	Vegetation; Developmen	Vascular Plants; <i>Gossypium</i> ; Species not re ht/Growth	eported in tex	t.; Embryo			
HERO ID:	1639289	ialate (DBP)					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.			
	Metric 2:	Test Substance Source	Low	The source of the DBP was not reported.			
	Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.			
Domain 2: Test Design							
Domani 2. Test Design	Metric 4:	Negative Controls	High	The study authors reported the use of a concurrent negative control and a solvent control with acetone.			
	Metric 5:	Negative Control Response	Medium	The negative control response was reported in Table 1. It is uncertain if the control re- sponse reported was the solvent control or not.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the cotton seeds were allocated into study groups.			
Domain 3: Exposure Ch	oracterization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details were provided on the test system and on the preparation of the test con- centrations. Exposure was conducted in glass Petri dishes.			
	Metric 8:	Consistency of Exposure	Low	Little details were provided on the exposure administration.			
	Metric 9:	Administration Measurement of Test Substance	Low	It was not reported if the test concentrations were measured at any point in the study.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration for the germination and root length portion of the study was reported to be 5 days. This was adequate to observe a response.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 10 exposure levels plus a negative control and a solvent control. Spacing appeared adequate to observe a response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported the use of acetone a vehicle solvent. The solvent control response was adequate.			
Domain 4: Test Organis	m						
2 children in rost organis	Metric 13:	Test Organism Characteristics	Low	The source of the cotton seeds was not reported. The scientific name was also not re- ported.			
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of cotton seeds used and the number of replicates was not reported.			
Domain 5: Outcome As	sessment	· · · · · · · · · · · · · · · · · · ·					

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Environmental Hazard Evaluation

HERO ID: 1639289 Table: 2 of 2

Study Citation: W	Vu. Y Yuan	S. I. (2012) Dibutyl phthelate pollution				
5.	Wu, Y., Yuan, S. L. (2012). Dibutyl phthalate pollution on cotton growth and physiological characteristics of cotton. Advanced Materials Research 518- 523:5436-5441					
Duration: C	23:5436-544 Overall Dura	tion: 4 - 10 days: Exposure Duration: 0 - 4	davs (0-96h)		
Exposure Route, T	errestrial; W	Vater; 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: V	Vegetation; Vascular Plants; Gossypium; Species not reported in text.; Embryo					
Health Outcome: D	Development/Growth					
Chemical: D	bibutyl phtha	alate (DBP)				
HERO ID: 14	639289					
Domain		Metric	Rating	Comments		
Μ	Ietric 16:	Adequacy of Test Conditions	Low	The environmental conditions were not sufficiently reported to evaluate if they were adequate for organism health.		
Ν	letric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-root length.		
Ν	Ietric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol were not reported.		
Domain 6: Confounding / V	ariable Con		Ŧ			
IV.	letric 19:	Confounding variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
N	Aetric 20.	Design and Procedures Outcomes Unrelated to Exposure	Medium	Conditions.		
14	ietrie 20.	Outcomes Oniciated to Exposure	Wiedrum	attrition or health outcomes unrelated to exposure.		
Domain 7: Data Presentatio	on and Analy	vsis				
N	fetric 21:	Statistical Methods	Low	Statistical analysis was performed but the methods used were not described.		
Ν	Ietric 22:	Reporting of Data	High	The control response and the exposure response for germination were reported in Table 1.		
Ν	Ietric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.		
Additional Comments: T	his portion	of the evaluation was on the effect of DBP	on the root l	ength of cotton seeds. Development/growth was selected as the outcome of interest.		

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Study Citation:	Dueck, T. A	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant					
Duration	species. Che	mosphere $53(8):911-920$.	1 dava				
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e.	r days chemical of i	nterest in exposure water, but unable to determine exact untake route)			
Media, Path:	Terrestriar, 7	in, not determined by study dunois (i.e.,	enemiear or i	nerest in exposure water, but diable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Holcus lanatus; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ADME (biotransformation)					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1302103						
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	Low	Purity or grade of test substance were not reported.			
Domain 2: Test Design							
Domain 2. Test Design	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.			
	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	haracterization	Engening and all Southerns /Teach Madia	II:-h				
	Metric 7:	Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum.			
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups based on timed sam- plings.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."			
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-			
		Spacing of Exposure Levels		tration gradient is reported as both actual and nominal within table 1.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.			
Domain 4. Test Organis	m						
Domain 4. 1050 Organis	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.			
		Cont	inued on nex	t page			

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation:	Dueck, T. A species. Che	., Dijk, Van, C. J., David, F., Scholz, N., V mosphere 53(8):911-920.	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant			
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; Holcus lanatus; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	ADME (biotransformation)						
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1302103						
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	Low	Four plants per treatment and no replicates reported, "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP.			
Domain 5: Outcome As	sessment	A damage of Track Conditions	II: -l-				
	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	Medium	Outcomes were assessed consistently across study groups but few details were reported.			
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.			
Domain 7. Data Pragant	ation and Anal	veic					
Domain 7. Data Present	Metric 21.	ysis 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			
	1710uic 22.	Reporting of Data	mgn	in Table 2.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.			
Additional Comments:	This form is	for ADME (biotransformation) assessmen	t of DBP con	centration in leaf tissue.			
0 110 11		•	TT+ P				
Overall Qualit	ty Detern	nination	High				

Overall Quality Determination

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

Study Citation:	Dueck, T. A species. Che	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.					
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; A	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; 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; Vascular Plants; <i>Holcus lanatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 1302103						
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	Low	Purity or grade of the test substance were not reported.			
Domain 2: Test Design							
2 china 21 1000 2 congu	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.			
	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 Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "bardened glass" and aluminum			
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups based on timed sam- plings.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."			
	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	Medium	The number of exposure groups and spacing of exposure levels were suitable. Concen- tration gradient is reported as both actual and nominal within table 1.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.			
Domain 4: Test Organis	m						
Domain 1. Tost Organis	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.			
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.			
	Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

		conti	nued from p	revious page				
Study Citation:	Dueck, T. A species. Che	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.						
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21	days					
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; Holcus lanatus; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1302103							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and Replicates per Group	Low	Four plants per treatment and no replicates reported, "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP."				
Domain 5: Outcome As	sassmant							
Domain 5. Outcome As	Matric 16:	A deguagy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism				
	Meule 10.	Adequacy of Test Conditions	mgn	health.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.				
	Metric 18:	Consistency of Outcome Assessment	Medium	Outcomes were assessed consistently across study groups but few details were reported.				
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:	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:	This form re	presents growth outcomes associated with	Dry Weight 1	reported for Holcus shoot and roots within Figure 3 on page 6/10.				

Overall Quality Determination

High

Study Citation: Duration: Exposure Route, Media Path:	Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serrulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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:	Vegetation; Vascular Plants; <i>Leptochloa chinensis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 5432995						
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. This may be an extracted plant extract for this test.			
	Metric 3:	Test Substance Purity	Uninformative	No information was provided on purity of the chemical which was an extracted fraction of plant material.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a 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, although the test concentration was not reported.			
	Metric 8:	Consistency of Exposure Administration	Medium	There was no mention of irregularities in exposure administration.			
	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	N/A	Only one treatment was tested.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	No concentration was reported.			
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 pretreatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Fifty seeds were used, but there were no replicates.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		С	ontinued on next page .				

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Environmental Hazard Evaluation

HERO ID: 5432995 Table: 1 of 1

		con	tinued from previou	s page			
Study Citation:	Chuah, T. S.	Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass					
	(Chrysopogo	(Chrysopogon serrulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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:	Vegetation;	Vascular Plants; Leptochloa chinensis; Not App	plicable (e.g., fungi or	algae studies) or Not Reported			
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5432995						
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: 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.			
	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					
Domain 7. Dua Presen	Metric 21	Statistical Methods	High	Statistical methods were adequately described			
	Metric 22:	Reporting of Data	Low	Control data was not presented			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes			
	infetite 25.		ingn	There were no unexpected outcomes.			
Additional Comments:	This is for t	ble 4 germination. This outcome was judged i	unaccentable because	the source of the exposure compound was unclear, and it appears to have			
raditional comments.	been applied	as a mixture of compounds derived from solv	ent extraction of a pla	nt homogenate. The exposure concentration is unknown			
	Jeen applied	as a mixture of compounds derived from sort	ent extraction of a pla	in noniogenate. The exposure concentration is anthrown.			

Overall Quality Determination

Uninformative

Dibutyl Phthalate

Study Citation:	Chuah, T. S.	, Oh, H. Y., Habsah, M., Norhafizah, M. Z.	, Ismail, B. S. (2	014). Potential of crude extract and isolated compounds from golden beard grass			
Duration: Exposure Route, Media Path	(Chrysopogo Overall Dura Terrestrial; S	on serrulatus) for control of sprangletop (Le ation: 11 - 21 days; Exposure Duration: 11 Soil; Not determined by study authors (i.e.,	ptochloa chinens - 21 days chemical of inter	is) in aerobic rice systems. Crop and Pasture Science 65(5):461-469. est in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation:	Vascular Plants: Leptochlog chinensis: Not	Applicable (e.g.,	fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	5432995	5432995					
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	PESTANAL analytic standard grade was used.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported.			
	Metric 6:	Randomized Allocation	Medium	"Each experiment was arranged in a completely randomized design with five replica- tions. Each experiment was repeated twice."			
Domain 2: Exposure Ch	aractorization						
Domain 5: Exposure Cr	Matria 7:	Experimental System/Test Madia	Low	The study merided only limited details on the macquires taken to emmoniotely menors			
	Metric 7.	Preparation	LOW	test concentrations.			
	Metric 8:	Consistency of Exposure	Medium	There was no mention of irregularities in exposure administration.			
	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 exposure frequency were reported and appropriate for the study type.			
	Metric 11:	Number of Exposure Groups/	High	Three treatments over an acceptable range were tested.			
	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	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 pretreatment conditions were the same for control and exposed groups.			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	"Each experiment was arranged in a completely randomized design with five replica- tions. Each experiment was repeated twice."			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Conti	nued on next pa	ge			

Environmental Hazard Evaluation

HERO ID: 5432995 Table: 1 of 1

		contin	ued from previ	ous page			
Study Citation:	Chuah, T. S. (Chrysopogo	Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.					
Duration:	Overall Dura	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Vegetation; V	Vegetation; Vascular Plants; Leptochloa chinensis; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5432995						
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, and data was pooled over			
		Assessment		several days of measurements.			
		rissessment					
Domain 6: Confoundir	g / Variable Cor	ntrol					
Domain 6: Confoundir	ng / Variable Con Metric 19:	ntrol Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental			
Domain 6: Confoundin	ng / Variable Con 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.			
Domain 6: Confoundir	ng / Variable Con 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.			
Domain 6: Confoundir Domain 7: Data Preser	ng / Variable Con Metric 19: Metric 20: ntation and Anal	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure vsis	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.			
Domain 6: Confoundir Domain 7: Data Preser	ng / Variable Con Metric 19: Metric 20: ntation and Anal Metric 21:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure ysis Statistical Methods	Low Medium Low	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. Statistical differences were not determined. Standard deviations of the mean were provided visually in Fig. 4.			
Domain 6: Confoundir Domain 7: Data Preser	ng / Variable Con Metric 19: <u>Metric 20:</u> ntation and Anal Metric 21: Metric 22:	ntrol Confounding Variables in Test Design and Procedures Outcomes Unrelated to Exposure ysis Statistical Methods Reporting of Data	Low Medium 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. Statistical differences were not determined. Standard deviations of the mean were provided visually in Fig. 4. Data was pooled for all stages of seedlings.			

Overall Quality Determination

Medium

Study Citation:	Ma, T., Teng Frontiers of I	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: Exposure Route,	Overall Dura Terrestrial; S	ition: 0 - 4 days (0-96h); Exposure Duratio oil; Not determined by study authors (i.e.,	n: 0 - 4 days chemical of	(0-96h) interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Lolium perenne; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical: HFRO ID:	2915866	alate (DBP)				
Domain	2713000	Metric	Rating	Comments		
Domain 1: Test Substance	ce		Tuning			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	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	High	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 adequate.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.		
Domain 3: Exposure Ch	aracterization					
2 onium of 2. posure on	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.		
	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 were exposed via soil.		
Domain 4: Test Organisr	n					
	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 Ass	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.		
		Conti	nued on nex	ct page		

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		contir	nued from p	revious page			
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,	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; Lolium perenne; Embryo						
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
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	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 I However, th growth in the	DEHP at a range of concentrations in the ex ey did exert effects on root elongation, se e evaluation of the phytotoxicity of PAE co	perimental s edling grow mpounds."	oil showed no discernible effect on the germination rate of the seven test plant species. th and biomass to different extents, indicating the potential applicability of seedling			
Overall Qualit	ty Determ	nination	High				

Dibutyl Phthalate

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 Mechanistic-Biomarkers (exposure and effect)-Photosynthesis Dibutyl phthalate (DBP) 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 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:	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 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:	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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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 2 of 3

		conti	nued from p	revious page			
Study Citation:	Ma, T., Teng Frontiers of	Ia, 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. rontiers 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;	Vegetation; Vascular Plants; Lolium perenne; Embryo					
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Dibutyl phth	nalate (DBP)					
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.			
	Metric 20:	Design and Procedures 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 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:	nts: This is for pigment content."The results indicate that longer periods of cultivation of the test plants may make it easier to interpret the changes in pigment contents."						
Overall Qualit	ty Deterr	nination	High				

Dibutyl Phthalate

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	Frontiers of E	Environmental Science & Engineering 9(2) tion: 0 - 4 days (0-96h): Exposure Duration	:259-268. n: 0 - 4 days	(0.96 b)		
Exposure Route.	Terrestrial: S	oil: Not determined by study authors (i.e.	chemical of	interest in exposure water, but unable to determine exact untake route)		
Media. Path:	Terrestria, 5	in, i tot determined by study damors (i.e.,	chemieur or			
Taxa, Species, Age:	Vegetation; V	ascular Plants; <i>Lolium perenne</i> ; Embryo				
Health Outcome:	Reproductive	/Teratogenic				
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	2915866					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	ce					
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.		
	Metric 2:	Test Substance Source	Low	Source was reported but he 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.		
Domain 2: Exposure Ch	aractorization					
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for propagation of test modes were described in		
	Mettic 7.	Preparation	nıgıı	adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
		Administration	8	, 6t.		
	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		
		Spacing of Exposure Levels	U	response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.		
Domain 4: Test Organist	n					
	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 observator		
	Methe 15.	Replicates per Group	Wiculum	ize toxicological effects.		
		Repleates 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.		
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.		
		Assessment		· · · · · ·		
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Dibutyl Phthalate

HERO ID: 2915866 Table: 3 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; 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:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	2915866						
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. 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:	This form re	presents the germination rate results preser	nted in Table	1 for Lolium perenne with DBP exposure.			
Overall Ouali	tv Deterr	nination	High				

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Study Citation:	Ma, T., Teng Frontiers of I	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: Exposure Route,	Overall Dura Terrestrial; S	ition: 0 - 4 days (0-96h); Exposure Duratio oil; Not determined by study authors (i.e.,	n: 0 - 4 days chemical of	(0-96h) interest in exposure water, but unable to determine exact uptake route)		
Media, Path: Taxa, Species, Age:	Vegetation; V	Vascular Plants; Medicago sativa; Embryo				
Health Outcome:	Developmen	t/Growth				
Chemical: HERO ID:	2915866	alate (DBP)				
Domain	2713000	Metric	Rating	Comments		
Domain 1: Test Substand	ce		<u> </u>			
	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 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 Administration	High	Exposures were administered consistently across study groups.		
	Metric 9:	Measurement of Test Substance 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/ 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 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	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					
2 small 5. Gutome 113	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.		
		Confi	nued on nev	at page		

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

continued from previous page					
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 Dibutyl phthalate (DBP) 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: "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 Qualit	ty Detern	nination	High		

Dibutyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Ma, T., Teng Frontiers of I Overall Dura Terrestrial; S Vegetation; V Mechanistic- Dibutyl phth 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>Medicago sativa</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosynthesis Dibutyl phthalate (DBP) 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						
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 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	8	response		
	Metric 12:	Testing at or Below Solubility Limit	N/A	seeds 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 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: 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; 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; Medicago sativa; Embryo						
Health Outcome:	Mechanistic	-Biomarkers (exposure and effect)-Photosy	nthesis				
Chemical:	Dibutyl phth	alate (DBP)					
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	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:	Pigment Cor	ntent"The results indicate that longer period	ds of cultivat	ion of the test plants may make it easier to interpret the changes in pigment contents."			
Overall Qualit	ty Detern	nination	High				

Dibutyl Phthalate

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	Frontiers of I	Environmental Science & Engineering $9(2)$:259-268. n: 0 - 4 days	(0.96 b)		
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:	i en estima, s					
Taxa, Species, Age:	Vegetation; V	/ascular Plants; <i>Medicago sativa</i> ; Embryo				
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	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	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 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	High	The avnarimental system and methods for preparation of test media were described in		
	Wieure 7.	Preparation	mgn	adequate detail.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	Matria Or	Administration	T			
	Metric 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 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	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		TT' 1			
	Metric 16:	Adequacy of Test Conditions	Hıgh	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.		
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Dibutyl Phthalate

		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; 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; Medicago sativa; Embryo						
Health Outcome:	Reproductiv	e/Teratogenic						
Chemical:	Dibutyl phth	nalate (DBP)						
HERO ID:	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:	This form re	presents the germination rate results presen	ited in Table	1 for Triticum medicago sativa with DBP exposure.				
Overall Quali	ty Deterr	nination	High					

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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						
Duration:	and seedling	growth. Pedosphere 27(6):1073-1082. tion: 4 - 10 days: Exposure Duration: 4 -	10 days				
Exposure Route,	Terrestrial; C	Cell Culture Media; 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:	Vegetation; V	/egetation; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5627041						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	Ce Matria 1:	Test Substance Identity	Low	Chamical was identified by nome only			
	Metric 1:	Test Substance Source	Low	Chemical was identified by name only The test substance identify was not analytically varified by the performing laboratory			
	Metric 3:	Test Substance Purity	High	Penorted as "guaranteed reagent grade"			
	Wiettie 5.	Test Substance Turity	Ingn	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 2. Euroques Ch	anastanization						
Domain 5. Exposure Ch	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to enpropriately pro			
	Metric 7.	Preparation	Low	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 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 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	C	response			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organisi	n						
0	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	-	· ·			
	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	inued on nex	xt page			

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HERO ID: 5627041 Table: 1 of 2

		conti	nued from p	revious page			
Study Citation:	Deng, J., Zha and seedling	ang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhan growth. Pedosphere 27(6):1073-1082.	g, S. (2017).	Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination			
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 1	10 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; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Reproductive/Teratogenic						
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	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 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	There was no information in the study to suggest 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	Unexpected outcomes were satisfactorily explained			
Additional Comments:	germination	effects					
Overall Qualit	ty Detern	nination	High				

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 speadling growth Badoghere 27(6):1073-1082							
Duration: Exposure Route, Media Path:	Overall Dura Terrestrial; C	tion: 4 - 10 days; Exposure Duration: 4 -	10 days dy authors (i.	e., chemical of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vegetation; V	/ascular Plants; <i>Nicotiana tabacum</i> ; cv K3	26; Not App	licable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Development	Jevelopment/Growth Dibutyl phthalate (DBP)						
HERO ID:	5627041	527041						
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	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 test substance was reported as "guaranteed reagent grade."				
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	Medium	The study reported that test containers were randomly distributed.				
Domain 2. Euroquina Ch	anastanization							
Domain 5: Exposure Ch	Motrio 7:	Experimental System/Test Media	Low	The study movided only limited details on the measures taken to encomistally me				
	Weule 7.	Preparation	Low	pare test concentrations. Authors reported using glassware: vials, funnels, bottles and beakers. No use of plastic vessels was 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 4: Test Organisi	Il Matria 12:	Test Organism Characteristics	Madium	The source of the coade was not reported				
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms				
	Methe 14.	Conditions	Ingn	An predeatment conditions were the same for condor and exposed organisms.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test plants was not reported, but three replicates were used.				
		Tephones per Group		-				
Domain 5: Outcome Ass	sessment							
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system (controlled chamber) 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.				
		Cont	inued on nev	t nage				

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Dibutyl 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					
Duration	and seedling	growth. Pedosphere $2/(6):10/3-1082$.	10 dava				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 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:	N 7 () ¹						
Taxa, Species, Age:	Vegetation;	Vascular Plants; <i>Nicotiana tabacum</i> ; cv K3	26; Not App	licable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5627041						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	y / 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	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:	This form is	for vigor index and length.					
Overall Qualit	ty Detern	nination	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	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 Developmen Dibutyl phth 792357	⁷ egetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) ¹ 92357						
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 and/or grade of the 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 that 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							
Domain 4. 10st Organisi	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				
		Conditions	2011	exposed groups.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates were not reported.				

Domain 5: Outcome Assessment

Continued on next page ...

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 1 of 4

continued from 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-				
Dermetterne	96.	96. June 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				
Duration:	Tormostrials X	uton: 4 - 10 days; Exposure Duration: 4 - 1	lu days	finterest in supscure water but unable to determine sweet untere route)		
Exposure Koute,	Terrestriar; v	water, not determined by study autions (i.e	., chemical c	of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:	N/		J. N. A.	nlinghle (n		
Taxa, Species, Age:	vegetation;	vascular Plants; <i>Nicotinana tobacum</i> ; Hong	g da; Not Apj	plicable (e.g., lungi of algae studies) of Not Reported		
Health Outcome:	Developmen	t/Growth				
Cnemical:	Dibutyl phth	alate (DBP)				
HERO ID:	192357					
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.		
Demein (. Cenferralia		-41				
Domain 6: Confounding	g / Variable Col	ntrol Conformationa Maniables in Tract	T			
	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 use no information in the study to success thifferences among around		
	Metric 20.	Outcomes Onierated to Exposure	Weuluili	There was no mormation in the study to suggest 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:	Growth (Dev	velopment-Slowed, Retarded, Delayed or N	on-developn	nent, Response Site: Not reported)		
Overall Qualit	ty Detern	nination	Low			

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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; Vascular Plants; <i>Nicotinana tobacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 792357					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	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 Cha	aracterization					
Ĩ	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 Organisr	n					
	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	essment					
	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	at page		

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 2 of 4

continued from 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-				
D (1	96. II D	96.				
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; V	Vater; Not determined by study authors (i.e	e., chemical c	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; <i>Nicotinana tobacum</i> ; G168	3; Not Applic	cable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
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		
		4 1				
Domain 6: Confounding	g / Variable Coi		T			
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental		
	M-4	Design and Procedures	Madian	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				
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 Chexpected Outcomes	111511			
Additional Comments:	Growth (Dev	velopment-Slowed, Retarded, Delayed or N	lon-developn	nent, Response Site: Not reported)		
Overall Qualit	ty Detern	nination	Low			

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HERO ID: 792357 Table: 3 of 4

07		ne substances	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-				
90. Duration: Overall Dr Exposure Route, Terrestrial Media Path:	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:VegetationHealth Outcome:ReproductChemical:Dibutyl phHERO ID:792357	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 792357						
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	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 Characterizatic	n						
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 Organism							
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 Assessment							
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	at page				

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Environmental Hazard Evaluation

HERO ID: 792357 Table: 3 of 4

continued from 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-				
Duration	96. Overall Dur	96. Overall Duration: 4 10 days, Exposure Duration: 4 10 days				
Exposure Route.	Terrestrial V	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 untake route)				
Media. Path:	Terrestitui, (refrestriat, water, not determined by study authors (i.e., enclinear of interest in exposure water, but unable to determine exact uptake route)				
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Nicotinana tobacum; G168	3; Not Applic	able (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Reproductive	e/Teratogenic				
Chemical:	Dibutyl phth	alate (DBP)				
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 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		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	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:	Germination					
Overall Quality Determination			Low			

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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: HERO ID:	Vegetation; Reproductiv Dibutyl phth 792357	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported Reproductive/Teratogenic Dibutyl phthalate (DBP) 792357					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ice						
	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	naracterization						
	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						
	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

continued from 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-				
Duration	96. Overall Duration: 4 10 days: Exposure Duration: 4 10 days					
Fynosure 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	referential, 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	Vascular Plants: Nicotinana tobacum: Hong	g da: Not Ap	plicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Reproductive	e/Teratogenic	,, . .			
Chemical:	Dibutyl phth	alate (DBP)				
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		
	· 1 • 1					
Domain /: Data Present	Matria 21.	ysis Statistical Mathada	Hish	Charles i an allo an an adamanta in da an inda		
	Metric 21: Metric 22:	Statistical Methods Reporting of Data	High	Statistical methods were adequately described		
	Metric 22.	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes		
	Wietric 25.	Explanation of Onexpected Outcomes	Ingn			
Additional Comments:	Germination					
Overall Quality Determination			Low			

Study Citation:	Isogai, Y., K	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth					
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; C	Serestrial; 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; V	egetation; Vascular Plants; Oryza sativa; cv Kotaketamanishiki; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	Development/Growth					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	5551990						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical name and structure were used to identify test chemical.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was not reported.			
Domain 2: Test Design							
2 onnani 21 Teor 2 eorgi	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.			
	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 test concentrations were not measured. Glass vessels were used.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	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 suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations but a wide range tested.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.			
Domain 4: Test Organis	m						
rest organis	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not reported.			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and			
		Conditions		exposed groups.			
	Metric 15:	Number of Organisms and	Low	25 seeds were tested per treatment but number of replicates was not reported			
		Replicates per Group					
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Number of plants per container and environmental conditions were not sufficiently re- ported to evaluate if 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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Dibutyl Phthalate

continued from previous page							
Study Citation:	Isogai, Y., K regulators. S	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.					
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; '	Vascular Plants; Oryza sativa; cv Kotaketam	anishiki; Not Aj	pplicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5551990						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.			
		. 1					
Domain 6: Confounding	g / Variable Col		T				
	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	Low	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	High	Results were reported in Table 2 for the control response and the exposure responses. The data was appropriate for the outcomes of interest			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.			
Additional Comments:	This portion	This portion of the study was on the plant growth promoting activity of DBP on Oryza sativa-cv Kotaketamanishiki. Development/Growth was selected as					

Overall Quality Determination

Medium

Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth						
Duration:	Overall Dura	Dverall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by stud	y authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Oryza sativa; cv Tanginboz	u; Not Applicabl	le (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5551990						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce		TT' 1				
	Metric 1:	Test Substance Identity	High	Chemical name and structure were used to identify test chemical.			
	Metric 2:	Test Substance Source	Low	The source was not reported.			
	Metric 5:	Test Substance Purity	Low	Purity or grade of test substance was 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.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.			
Domain 2. Evenagues Ch	anastanization						
Domain 5: Exposure Ch	Matria 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to encounistally propose			
	Wietric 7.	Preparation	LOw	test concentrations and test concentrations were not measured. Glass vessels were used			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	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 suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations but a wide range tested.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.			
Domain 4: Test Organisi	m Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not reported			
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and			
	Metrie 11.	Conditions	Low	exposed groups.			
	Metric 15:	Number of Organisms and	Low	25 seeds per treatment but number of replicates was not reported.			
		Replicates per Group					
Demain 5. Outerman Ar							
Domain 5: Outcome Ass	Metric 16	A deguage of Test Conditions	Low	Number of plants per container and any ironmental conditions were not sufficiently a			
	Metric 10.	Adequacy of Test Conditions	Low	ported 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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.			
		Conti	nued on next pa	ge			
Continued on next page							

Dibutyl Phthalate

continued from previous page						
Study Citation:	Isogai, Y., K regulators. S	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education. University of Tokyo 22(2):129-135.				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., cl	nemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation;	Vascular Plants; Oryza sativa; cv Tanginbozu	u; Not Applicab	le (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5551990					
Domain		Metric	Rating	Comments		
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	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	High	Results were reported in Table 2 for the control response and the exposure responses. The data was appropriate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion	This portion of the study was on the plant growth promoting activity of DBP on Oryza sativa-cv Tanginbozu. Development/Growth was selected as the outcome of interest				

Overall Quality Determination

Medium

Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135.					
Duration: Exposure Route, Media, Path:	Overall Dura Terrestrial; C	Perrestrial; 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; V	Vascular Plants; Oryza sativa; cv Norin #29	; Not Applicable	(e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical: HERO ID:	Dibutyl phth 5551990	alate (DBP)				
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1:	Test Substance Identity	High	Chemical name and structure were used to identify the test chemical.		
	Metric 2:	Test Substance Source	Low	The source was not reported.		
	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 a 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					
Ĩ	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare		
		Preparation		test concentrations and test concentrations were not measured. Glass vessels were used.		
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.		
	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 suitable for the study type.		
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations were used, but a wide range was tested.		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.		
Domain 4: Test Organisi	m Matria 12:	Test Organism Characteristics	Low	The source of the test could use not reported		
	Metric 15:	Acclimatization and Pretreatment	Low	The source of the test seeds was not reported.		
	Methe 14.	Conditions	Low	exposed groups.		
	Metric 15:	Number of Organisms and	Low	There were 25 seeds tested per treatment, but the number of replicates was not reported.		
		Replicates per Group				
Domain 5. Outcome Ass	Metric 16:	Adequacy of Test Conditions	Low	Number of plants per container and environmental conditions were not sufficiently re- ported 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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.		
	Continued on next page					

Dibutyl Phthalate

		contin	ued from previ	ous page	
Study Citation:	Isogai, Y., K	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth			
Duration:	Overall Dura	ation: 4 - 10 days: Exposure Duration: 4 - 10) davs	Ský 01 10ký0 22(2).12) 155.	
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:			× ,		
Taxa, Species, Age:	Vegetation;	Vascular Plants; Oryza sativa; cv Norin #29;	Not Applicable	(e.g., fungi or algae studies) or Not Reported	
Health Outcome:	Developmen	t/Growth			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	5551990				
Domain		Metric	Rating	Comments	
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	tation and Anal	veie			
Domain 7. Data Presen	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted	
	Metric 22:	Reporting of Data	High	Results were reported in Table 1 for the control response and the exposure responses.	
	Matric 22.	Explanation of Unexpected Outcomes	High	I ne data was appropriate for the outcomes of interest.	
	Meute 23:	Explanation of Onexpected Outcomes	підп	Study autnors and not report any unexpected outcomes.	
Additional Comments:	This portion outcome of i	This portion of the study was on the plant growth promoting activity of DBP on Oryza sativa-cv Norin #29. Development/Growth was selected as the outcome of interest.			

Overall Quality Determination

Medium

Study Citation:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education. University of Tokyo 22(2):129-135.						
Duration:	Overall Dura	Dverall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days					
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., ch	emical of interest in exposure water, but unable to determine exact uptake route)			
Media, Path:							
Taxa, Species, Age:	Vegetation; V	ascular Plants; <i>Oryza sativa</i> ; cv Norin #22;	Not Applicable	(e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Developmen	t/Growth					
Chemical:	5551000	alate (DBP)					
	5551990						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	Metric 1:	Test Substance Identity	High	Chamical name and structure were used to identify test chamical			
	Metric 2:	Test Substance Source	Low	The source was not reported			
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was not reported			
			2011				
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.			
	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	Low	The study provided only limited details on the measures taken to appropriately prepare			
	Weute 7.	Preparation	Low	test concentrations and test concentrations were not measured. Glass vessels were used.			
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.			
	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 suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations but a wide range tested.			
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.			
Domain 4: Test Organisr	n		т				
	Metric 13: Matria 14:	A colimatization and Protrootmont	Low	The source of the test seeds was not reported.			
	Meure 14.	Conditions	Low	exposed groups.			
	Metric 15:	Number of Organisms and	Low	25 seeds were tested per treatment but number of replicates was not reported.			
		Replicates per Group					
Domain 5: Outcome A	acomart						
Domain 5: Outcome Ass	Metric 16	A dequacy of Test Conditions	Low	Number of plants per container and environmental conditions were not sufficiently re-			
	Meure 10.	Adequacy of Test Conditions	Low	ported 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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.			
Continued on next page							

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Dibutyl Phthalate

continued from previous page					
Study Citation:	Isogai, Y., K	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth			
Destin	regulators. S	cientific papers of the College of General Ec	lucation, Univer	rsity of Tokyo 22(2):129-135.	
Duration:	Overall Dura	ation: 4 - 10 days; Exposure Duration: 4 - 10	days		
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)	
Media, Path:					
Taxa, Species, Age:	Vegetation;	Vascular Plants; Oryza sativa; cv Norin #22;	Not Applicable	(e.g., fungi or algae studies) or Not Reported	
Health Outcome:	Developmen	t/Growth			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	5551990				
Domain		Metric	Rating	Comments	
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.	
	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	Low	Statistical analysis was not conducted.	
	Metric 22:	Reporting of Data	High	Results were reported in Table 1 for the control response and the exposure responses. The data was appropriate for the outcomes of interest.	
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.	
Additional Comments:	This portion of the study was on the plant growth promoting activity of DBP on Oryza sativa-cv Norin #22. Development/Growth was selected as the outcome of interest.				

Overall Quality Determination

Medium

Study Citation: Duration: Exposure Route, Media Path:	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth regulators. Scientific papers of the College of General Education, University of Tokyo 22(2):129-135. 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: Health Outcome: Chemical: HERO ID:	Vegetation; V Development Dibutyl phthe 5551990	/ascular Plants; <i>Oryza sativa</i> ; cv Kinmase; N t/Growth alate (DBP)	Not Applicable (e.g., fungi or algae studies) or Not Reported	
Domain		Metric	Rating	Comments	
Domain 1: Test Substand	ce	· · ·			
	Metric 1:	Test Substance Identity	High	Chemical name and structure were used to identify test chemical.	
	Metric 2:	Test Substance Source	Low	The source was not reported.	
	Metric 3:	Test Substance Purity	Low	Purity or grade of test substance was not reported.	
Domain 2: Test Design					
2 onnani 21 Tese 2 osign	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.	
	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 test concentrations were not measured. Glass vessels were used.	
	Metric 8:	Consistency of Exposure	Low	The study provided few details on exposure administration.	
	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 suitable for the study type	
	Metric 11:	Number of Exposure Groups/	Medium	Only three concentrations but a wide range tested	
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.	
		5		x	
Domain 4: Test Organisi	n				
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not reported.	
	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	25 seeds were tested per treatment but number of replicates was not reported.	
Domain 5: Outcome Ass	sessment				
	Metric 16:	Adequacy of Test Conditions	Low	Number of plants per container and environmental conditions were not sufficiently re- ported 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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited.	
Continued on next page					

Dibutyl Phthalate

continued from previous page						
Study Citation:	Isogai, Y., K regulators, S	Isogai, Y., Komoda, Y., Okamoto, T. (1972). Biological activities of n-butyl phthalate and its analogous compounds on various bioassays of plant growth				
Duration:	Overall Dura	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days				
Exposure Route,	Terrestrial; C	Cell Culture Media; Not determined by study	authors (i.e., ch	nemical of interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation; Y	Vascular Plants; Oryza sativa; cv Kinmase; N	Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5551990					
Domain		Metric	Rating	Comments		
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.		
Domain 7: Data Present	tation and Anal	ysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	High	Results were reported in Table 1 for the control response and the exposure responses. The data was appropriate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.		
Additional Comments:	This portion outcome of i	This portion of the study was on the plant growth promoting activity of DBP on Oryza sativa-cv Kinmase. Development/Growth was selected as the outcome of interest				

Overall Quality Determination

Medium

Study Citation:	Chuah, T. S.	Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass				
Duration: Exposure Route,	Overall Dura Terrestrial; S	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)				
Media, Path: Taya Species Age:	Vegetation:	Vagetation: Vaccular Plante: Oruza sativa: Not Applicable (e.g., fungi or algae studies) or Not Perperted				
Health Outcome:	Developmen	t/Growth	ie (e.g., fuligi of	argae studies) of Not Reported		
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5432995					
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	PESTANAL analytic standard grade was used.		
Domain 2: Test Design						
C	Metric 4:	Negative Controls	High	Study authors reported using a 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 2: Exposure Ch	aractorization					
Domain 5: Exposure Ch	Matria 7	Experimental System/Test Media	Low	The study may ided only limited details on the macaynes taken to annomistally manage		
	Methe 7.	Preparation	LOw	test concentrations.		
	Metric 8:	Consistency of Exposure	Medium	There was no mention of irregularities in exposure administration.		
	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 exposure frequency were reported and appropriate for the study type		
	Metric 11:	Number of Exposure Groups/	N/A	Three treatments over an acceptable range were tested.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.		
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	Low	The study did not report whether pretreatment conditions were the same for control and		
		Conditions		exposed groups.		
	Metric 15:	Number of Organisms and	Low	There was one rice seed per cup, with five replicates per exposure, and the experiment		
		Replicates per Group		was repeated twice.		
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	High	The outcome assessment methodology reported the intended outcome of interest.		
Continued on next page						

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Environmental Hazard Evaluation

HERO ID: 5432995 Table: 1 of 1

	continued from previous page					
Study Citation:	Chuah, T. S. (Chrysopogo	Chuah, T. S., Oh, H. Y., Habsah, M., Norhafizah, M. Z., Ismail, B. S. (2014). Potential of crude extract and isolated compounds from golden beard grass (Chrysopogon serulatus) for control of sprangletop (Leptochloa chinensis) in aerobic rice systems. Crop and Pasture Science 65(5):461-469.				
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; S	Soil; 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:	Vegetation;	Vascular Plants; Oryza sativa; Not Applicab	ole (e.g., fungi or a	lgae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5432995					
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	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	tation and Anal	ysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.		
	Metric 22:	Reporting of Data	Medium	Data was pooled for application timing of seedlings.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This form is	for Table 5.				
Overall Quali	ty Detern	nination	Medium			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

 species. Chemosphere 53(8):911-920. Overall Duration: > 21 days Terrestrial; Air: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Wethi, Pathi Trenstrial; Air: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Wethi, Pathi Desclopment/Growth Divury I phthalate (DBP) HERO ID: 1302103 Metric 1: Test Substance Source Metric 2: Test Substance Source Liw The chemical was identified by name and CAS⁹. Metric 2: Test Substance Source Liw The chemical was identified by name and CAS⁹. Metric 2: Test Substance Source Liw The tots substance were not reported in gath concentrations are reported in all controls had some DBP detected. Control group, note that all controls had some DBP detected. Control group was reported and reasonable for assessed outcomes. Metric 6: Randomized Allocation Low Rescurcher did not report how organisms were allocated to study groups. Concentration Metric 9: Metric 10: Exposure Characterization Metric 9: Metric 9: Metric 10: Exposure Groupsi and propriate constructed of Takened gatas and propriate analytical technologies. Samples from each function groupsi and groupsi and groupsi anding and prop	Study Citation:	Dueck, T. A	., Dijk, Van, C. J., David, F., Scholz, N.,	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant		
Duration: > 21 days: Exposure Duration: = 21 days: = 21		species. Che	species. Chemosphere 53(8):911-920.				
Exposure Konce, Terestrait; Arr. Solt defermine in y study autinos (i.e., chemical of interest in exposure water, but uname to defermine exact uptake route) Weidin, Pathi Errestrait; Arr. Solt defermine by study autinos (i.e., chemical of interest in exposure water, but uname to defermine exact uptake route) Bins, Species, Age: Vegetation; Vascular Plants; Phaseablas valgaris; Not Applicable (e.g., fungi or algae studies) or Not Reported Domain Development/Growth Domain Metric 1: Test Substance Domain 2: Test Substance Identity High Metric 2: Test Substance Identity High The chemical was identified by name and CAS#. Domain 2: Test Substance Purity Low The test substance were nor reported. Domain 2: Test Substance Purity Low Study authors reported using an appropriat concurrent negative control group, note shat and characterization Metric 5: Negative Controls Low Study authors reported using an appropriat concurrent negative control group, note shat and allocation The biological response of the negative control group was reported an reasonable for reserved outcomes. Metric 5: Negative Control Response High Preparation The experimental system and methods for preparation of test media ware described in adequate deall. The chanhers were adinstaterd coundy group based on tinned sam- plings. </th <th>Duration:</th> <th>Overall Dura</th> <th colspan="5">Overall Duration: > 21 days; Exposure Duration: > 21 days</th>	Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days				
Water, and Trans, Species, Age: Vegetation: Vascular Plants; Phaseolas valgaris; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Divelopment/Growth Divelopment/Growth Divelopment/Growth Divelopment/Growth Domain 1: Test Substance Metric (BP) Domain 1: Test Substance Metric 1: Test Substance fearity High High The chemical was identified by name and CAS# Domain 2: Test Substance Former Metric 2: Test Substance Former Low Purity or grade of the test substance were not reported. Domain 2: Test Design Metric 4: Metric 4: Negative Controls Low Study authors reported using an appropriate concurrent negative control group parts econcentrations are reported in Table 1. 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 High The experimental system and methods for proparation of test media were described in adequate deal. The charabers were constructed of study groups have on timed sam- plings. Metric 10: Exposure Duration and Frequency High High The experimental system and methods for proparation of test media were described in adequate deal. The charabers were constructed of study groups based on time sam- plings. Metric 10: Exposure Duration and Frequency	Exposure Route, Modia Pathy	Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
 Test Design Development/Growth Development/Growth Detry phthalate (DBP) HERO ID: 130203 Domain Metric 1: Test Substance Identity Metric 2: Test Substance Purity Domain 1: Test Substance Purity Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Control Response High The experimental system and methods for preparation of test methods for preparation of test methods for association of the standard design of the standard des	Tava Snecies Age	Vegetation: V	Vascular Plants: Phaseolus vulgaris: Not A	Annlicable (e	g fungi or algae studies) or Not Reported		
Chemical: Dibutyl phthalate (DBP) 1302103 Domain 1: Test Substance Metric 1: Test Substance Identity Metric 2: Test Substance Metric 2: Test Substance Identity Metric 3: Test Substance Purity Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation Metric 6: Randomized Allocation Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Administration Metric 9: Metric 9: Metric 10: Experimental System/Test Media Preparation Metric 9: Metric 9: Metric 10: Experimental System/Test Media Preparation Metric 9: Metric 10: Exposure for Substance Metric 10: Exposure Of Substance Metric 10: Exposure of Test Substance Metric 10: Exposure Other and Substance Metric 10: Exposure Other and Substance Metric 10: Exposure Other and Substance Metric 10: Exposure of Test Substance Concentration Metric 10: Exposure Other and Substance Concentration Metric 10: Exposure Other and Substance Concentration Metric 10: Exposure Other of Test Substance Concentration Metric 11: Number of Exposure Group/ Metric 12: Test Organism Metric 12: Test Organism Metric 13: Test Organism Metric 13: Test Organism Metric 14: Test Organism Metric 15: Test Organism Metric 15: Test Organism Metric 16: Exposure Group/ Spacing of Exposure Concentration Spacing of Exposure Levels Metric 12: Test Organism Metric 13: Test Organ	Health Outcome:	Developmen	t/Growth	ipplicable (e.	g, rungi or uigue studies) or not reported		
HERO ID: 1302/03 Domain Metric Rating Comments Domain 1: Test Substance 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. Domain 2: Test Design Metric 4: Negative Controls Low Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group was reported in Table 1. Metric 5: Negative Control Response High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Domain 3: Exposure Characterization Low Researchers did not report how organisms were allocated to study groups. Domain 3: Exposure Characterization Low High The experimental system/Test Media Preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Metric 9: Metric 1: Resurrenent of Test Substance Concentrations were measured by taking air samples from each chamber using an automatel gas sampling device (GS-1 gas sam-plencGorstell AP exposure Concentrations were measured by taking air samples. For concentration were measured vising an audytical technologies. Sam-plencGorstell AP ethonesh	Chemical:	Dibutyl phth	alate (DBP)				
Domain Metric Rating Comments Domain 1: Test Substance Test Substance Identity Metric 2: Test Substance Source Test Substance Source Metric 3: High Tects substance identify was not analytically verified by the performing laboratory. Purity or 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. note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1. 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 9: Experimental System/Test Media Preparation Metric 9: High Metric 10: The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Administration Administration High Preparation Metric 9: Sposure Constructorization Metric 9: High Metric 10: Exposure constructed of "hardened glass" and aluminum. Concentrations were measured by taing any sumples from each chamber using appropriate analytical technologies. Sam- plenge. Metric 11: Number of Exposure Sposure Duration and Frequency High Metric 12: Exposure Concentrations were neasured by taing ari- sumples from each chamber using an automated gas sampling device (Gs-1 gas sam- sumples from each chamber using an automated gas sampling device (Gs-1 gas sam- sumples from each chamber using anoutomated gas sampling device (Gs-1 gas sam- sumples Group each (Gf	HERO ID:	1302103					
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Metric 1: Test Substance lidentity High The feest aubtance including of the performing laboratory. Metric 2: Metric 2: Test Substance Purity Low Purity or grade of the test substance were not reported. Domain 2: Test Design Metric 4: Negative Controls Low Metric 5: Negative Control Response High The hiological response of the negative control group. DBP concentrations are reported in Table 1. 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 Metric 9: Meaurement of Test Substance High The exposure concentrations were measured using an garpropriate analytical technologies. Samples, Concentration Metric 10: Exposure Duration and Frequency High The ecoposure ordenations were reassured using any appropriate analytical technologies. Samples, Concentration Metric 10: Exposure Duration and Frequency High The duration of exposure period (76 days). ² Metric 11: Number of Exposure Groups/ Metric 11: Number of Exposure Groups/ Metric 12: Test organism Metric 12: Test organism Characteristics Metric 12: Test organism Metric 12: Test organism Characteristics M	Domain 1: Test Substan	ce					
Metric 2: Test Substance Source Low The test substance dentity was not analytically verified by the performing taboratory. Domain 2: Test Design Metric 4: Negative Controls Low Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1. Metric 5: Negative Control Response High The biological response of the negative control group was reported and reasonable for assessed outcomes. 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 High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "handened glass" and aluminum. Metric 9: Mesurement of Test Substance High Exposure concentrations were measured using appropriate analytical technologies. Samples form cach chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)." Metric 10: Exposure Duration and Frequency High The duration of exposure addy or exposure levels were estable. Concentration were spacing of Exposure Levels Metric 11: Number of Exposure Duration and Frequency High The duration of exposure addy or exposure levels were estable. Concentration of exposure levels were estable in adi		Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.		
Metric 3: Test Substance Putry Low Punity or 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, note that all controls had some DBP detected. 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 Characterization Metric 7: Experimental System/Test Media Preparation High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Exposure Characterization Metric 9: Metric 10: Exposure Duration and Frequency High The under frequency'. Advantal DBP concentrations were reported using an unomated gas sampling device (GS-1 gas samples from each chamber was ganpling was performed with Tenax TA tubes (2-6 dipherylphenylphenylene vice dip day). ¹ 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 The number of exposure and/or exposure frequency were reported and a		Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.		
Domain 2: Test Design Metric 4: Negative Controls Low Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1. 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 Characterization Metric 7: Experimental System/Test Media Preparation High Metric 8: Consistency of Exposure Administration High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Metric 9: Measurement of Test Substance Concentration High Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automade gas sampling device (OS-1 gas sample frequently: "Decourted with Teax TA tubes (2.6-dipher/phere/tea co/sol epo/sure 12: 20ml min 16 r20 min. During the fumigation exposure add/or exposure frequently: "The duration of exposure add/or exposure frequency were reported and appropriate for the study type. Metric 10: Exposure Duration and Frequency High The duration of exposure frequency were reported and ap		Metric 3:	Test Substance Purity	Low	Purity or grade of the test substance were not reported.		
Metric 4: Negative Controls Low Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1. 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 Characterization Metric 7: Experimental System/Test Media Preparation High Metric 9: Consistency of Exposure Administration High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Metric 9: Measurement of Test Substance Consistency of Exposure administration High Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequenty: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampled in duplicate on a weekly basis during the complete exposure primerined, each chamber using an automated gas sampled in duplicate on a weekly basis during the complete exposure provise and/or exposure frequency were reported and appropriate for the study type. Metric 10: Exposure Duration and Frequency High The number of exposure groups and spacing of exposure foroups/ Spacing of Exposure foroups/ Spacin	Domain 2. Test Design						
Metric 5: Negative Control Response High Metric 6: The biological response of the negative control group was reported and reasonable for assessed outcomes. Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Metric 8: High Consistency of Exposure Administration High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Metric 9: Measurement of Test Substance Concentration High Metric 9: Exposure concentrations were measured using appropriate analytical technologies. Sam- ples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sam- ples were taken frequently: "Actual DBP concentrations were reported and appropriate analytical technologies. Sam- ples were taken frequently: "Actual DBP concentrations were neasured by taking air samples from each chamber using an automated gas sampling the fumiga- tion experiment, each chamber using an automated gas sampling the fumiga- tion experiment, each chamber using an automated gas sampling the fumiga- tion experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)." Metric 10: Exposure Coups/ Spacing of Exposure Groups/ Spacing of Exposure Cevels Medium N/A The unmber of exposure groups and spacing of exposure levels were suitable. Concen- tion strain gradient is reported as bot actual and nominal within table 1. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source	Domain 2. Test Design	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.		
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. The chambers were constructed of "hardned glass" and aluminum. Metric 8: Consistency of Exposure Administration High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardned glass" and aluminum. Metric 9: Measurement of Test Substance Concentration High Exposure concentrations were measured using appropriate analytical technologies. Sam- ples were taken frequenty: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sam- pler.Gerstel,M Eulheim, Germany). Sampling was performed with Tenax TA tubes (2.6-dipherylpherylene oxide oplumer) at 250ml minl for 20 min. During the furniga- tion experiment, each chamber was sampled in duplicate on a weekly basis during the complet exposure period (76 days)." Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Medium The number of exposure frequency were reported and appropriate for the study type. Oomain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.		
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Metric ?: Experimental System/Test Media High The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum. Metric 8: Consistency of Exposure High Exposure constructed of "hardened glass" and aluminum. Metric 9: Measurement of Test Substance High Exposure concentrations were measured using appropriate analytical technologies. Samplers. Metric 9: Measurement of Test Substance High Exposure concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler.Gerstel.M @Ulheim, Germany). Sampling was performed with Teax TA tubes (2.6-diphenylphenylene oxide polymer) at 250ml min 1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure prof (76 days)." Metric 10: Exposure Duration and Frequency High The duration of exposure frequency were reported and appropriate for the study type. Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Medium The number of exposure groups and spacing of exposure levels were suitable 1. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.	Domain 3: Exposure Ch	aracterization		TT' 1			
Metric 8: Consistency of Exposure Administration High Exposures were administered consistently across study groups based on timed sam- plings. Metric 9: Measurement of Test Substance Concentration High Exposure concentrations were measured using appropriate analytical technologies. Sam- ples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sam- pler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min I for 20 min. During the fumiga- tion experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)." Metric 10: Exposure Duration and Frequency High Metric 11: Number of Exposure Groups/ Spacing of Exposure Groups/ Spacing of Exposure Levels Medium Metric 12: Test Organism N/A Exposure was via fumigation in a continually renewed chamber. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric /:	Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum.		
Metric 9: Measurement of Test Substance Concentration High Exposure concentrations were measured using appropriate analytical technologies. Samples from each chamber using an automated gas sampling device (GS-1 gas sampler, Gerstel, M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumiga- tion experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)." Metric 10: Exposure Duration and Frequency High Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Medium Metric 12: Testing at or Below Solubility Limit N/A Domain 4: Test Organism Metric 13: Test Organism Characteristics Low Continue there Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups based on timed sam- plings.		
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 The number of exposure groups and spacing of exposure levels were suitable. Concentration gradient is reported as both actual and nominal within table 1. Metric 12: Testing at or Below Solubility Limit N/A Exposure was via fumigation in a continually renewed chamber. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."		
Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Medium The number of exposure groups and spacing of exposure levels were suitable. Concen- tration gradient is reported as both actual and nominal within table 1. Metric 12: Testing at or Below Solubility Limit N/A Exposure was via fumigation in a continually renewed chamber. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.		
Spacing of Exposure Levels tration gradient is reported as both actual and nominal within table 1. Metric 12: Testing at or Below Solubility Limit N/A Exposure was via fumigation in a continually renewed chamber. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 11:	Number of Exposure Groups/	Medium	The number of exposure groups and spacing of exposure levels were suitable. Concen-		
Metric 12: Testing at or Below Solubility Limit N/A Exposure was via fumigation in a continually renewed chamber. Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.			Spacing of Exposure Levels		tration gradient is reported as both actual and nominal within table 1.		
Domain 4: Test Organism Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.		
Metric 13: Test Organism Characteristics Low The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.	Domain 1: Test Organis	m					
	Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.		
Continued on next nage			Cont	inued on nev	at nage		

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 1

		conti	nued from p	previous page		
Study Citation:	Dueck, T. A species. Che	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.				
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days					
Exposure Route,	Terrestrial; Air; 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>Phaseolus vulgaris</i> ; Not A	pplicable (e.	g., fungi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth	II	6, , , , , , , , , , , , , , , , , , ,		
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	1302103					
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	Low	There were four plants per treatment and no replicates reported. "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP."		
Domain 5. Outcome As	acamant					
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.		
	Metric 18:	Consistency of Outcome Assessment	Medium	Outcomes were assessed consistently across study groups but few details were reported.		
Domain 6: Confounding	y / Variable Cou	ntrol				
Domain of Comountaing	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	veis				
Domain 7. Duta i losofit	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	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	This form re	presents growth outcomes associated with	- Dry Weight	reported for Phaseolus shoot and roots within Figure 3 on page 6/10.		
Overall Qualit	ty Detern	nination	High			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant					
Duration: Exposure Route, Media. Path:	species. Che Overall Dura Terrestrial; A	species. Chemosphere 53(8):911-920. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Air; 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; <i>Picea abies</i> ; Not Applicab	ole (e.g., fung	i or algae studies) or Not Reported		
Health Outcome:	ADME (biot	transformation)				
Chemical: HERO ID:	Dibutyl phth 1302103	alate (DBP)				
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	Purity or grade of the test substance were not reported.		
Domain 2: Test Design						
C C	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.		
	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 9: Exposure on	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum.		
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups based on timed sam- plings.		
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."		
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-		
	Matria 12:	Spacing of Exposure Levels	NI/A	tration gradient is reported as both actual and nominal within table 1.		
	Metric 12:	resung at or Below Solubility Limit	IN/A	Exposure was via lumigation in a continually renewed chamber.		
Domain 4: Test Organis	m					
2 chain in rest organis	Metric 13:	Test Organism Characteristics	Medium	The source of the test organisms (Picea abies) was from a local nursery. The crop and herbaceous species originated from germinated seeds.		
		Cont	inued on nex	xt page		

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 2

		conti	nued from p	revious page					
Study Citation:	Dueck, T. A	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920							
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	davs						
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; <i>Picea abies</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	ADME (biot	ADME (biotransformation)							
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	1302103								
Domain		Metric	Rating	Comments					
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.					
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were four plants per treatment and no replicates reported. "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP."					
Domain 5: Outcome A	ssassmant								
Domain 5. Outcome A	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	Medium	Outcomes were assessed consistently across study groups but few details were reported.					
Domain 6: Confoundin	og / Variable Cou	ntrol							
Domain 0. Comountum	Metric 19.	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions					
	Wieure 17.	Design and Procedures	mgn	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 Presen	tation and Anal	veie							
Domain 7. Data Flesen	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 in Table 2.					
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.					
Additional Comments:	This form is	for ADME (biotransformation) assessmen	t of DBP con	centration in leaf tissue.					

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

Study Citation:	Dueck, T. A	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant						
Duration: Exposure Route, Media, Path:	overall Dura Terrestrial; A	Solution: > 21 days; Exposure Duration: > 21 days Perrestrial; Air; 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; V Developmen	Vegetation; Vascular Plants; <i>Picea abies</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth						
Chemical: HERO ID:	Dibutyl phth 1302103	Dibutyl phthalate (DBP) 1302103						
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	Low	Purity or 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, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.				
	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.				
Damain 2. Enna ann Ch								
Domain 3: Exposure Ch	aracterization	Environmental Surdaux/Teach Madia	II: -1-					
	Metric 7:	Brongration	nign	a ne experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum				
	Metric 8.	Consistency of Exposure	High	Exposures were administered consistently across study groups based on timed sam-				
	Methe 0.	Administration	mgn	plings.				
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."				
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen- tration gradient is reported as both actual and nominal within table 1				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.				
	· · · ·							
Domain 4: Test Organis	m							
	Metric 13:	Test Organism Characteristics	Medium	The source of the test organisms (Picea abies) was from a local nursery. The crop and herbaceous species originated from germinated seeds.				
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.				
	Continued on next page							

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PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

		contii	nued from p	revious page					
Study Citation:	Dueck, T. A. species. Che	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.							
Duration:	Overall Dura	Overall Duration: > 21 days; Exposure Duration: > 21 days							
Exposure Route,	Terrestrial; Air; 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; Picea abies; Not Applicabl	le (e.g., fung	i or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth							
Chemical:	Dibutyl phth	alate (DBP)							
HERO ID:	1302103								
Domain		Metric	Rating	Comments					
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were four plants per treatment and no replicates reported. "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum num- ber physically possible) for exposure to DBP."					
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 Assessment	Medium	Outcomes were assessed consistently across study groups, but few details were reported.					
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:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among 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	Unexpected outcomes were satisfactorily explained.					
Additional Comments:	This form represents growth outcomes associated with Dry Weight reported for Picea within Figure 3 on page 6/10.								

Overall Quality Determination

High

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·		\mathcal{L} , \mathcal{L}	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant				
	species. Che	mosphere 53(8):911-920.						
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	l days chomical of i	ntarast in avnasura watar, but unable to datarmine avaat unteka routa)				
Exposure Koute, Media Path.	refrestriat, Air, Not determined by study autions (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Taxa. Species. Age:	Vegetation: V	Vegetation: Vascular Plants: <i>Plantago major</i> : Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Developmen	t/Growth	(8-,-					
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	1302103							
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	Purity or grade of the test substance were not reported.				
Domain 2. Test Design								
Domain 2. Test Design	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.				
	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		TT' 1					
	Metric /:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. The chambers were constructed of "hardened glass" and aluminum.				
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups based on timed sam- plings.				
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."				
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-				
	10.000	Spacing of Exposure Levels	3	tration gradient is reported as both actual and nominal within table 1.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.				
Domain 4. Test Organist	m							
Bomain 4. Test Organisi	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.				
		Cont	inued on nev	t nage				

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 1

		conti	nued from p	revious page			
Study Citation:	Dueck, T. A., Dijk, Van, C. J., David, F., Scholz, N., Vanwalleghem, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant species. Chemosphere 53(8):911-920.						
Duration:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., o	chemical of i	nterest in exposure water, but unable to determine exact uptake route)			
Media. Path:							
Taxa, Species, Age:	Vegetation: Vascular Plants: <i>Plantago major</i> : Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Development/Growth						
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1302103						
	1502105						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	Hıgh	The test organisms were acclimatized to test conditions.			
	Metric 15	Conditions Number of Organisms and	Low	There were four plants per treatment and no replicates reported. "Three to four weeks			
	Wieute 15.	Replicates per Group	LOW	after sowing when the first mature leaves appeared four uniform individuals per species			
		Replicates per Gloup		including the tree species were placed in each fumigation chamber (the maximum num-			
				ber physically possible) for exposure to DBP."			
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	Medium	Outcomes were assessed consistently across study groups but few details were reported.			
		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	Medium	There was no information in the study to suggest 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 22.	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained			
	Wietrie 25.	Explanation of Onexpected Outcomes	Ingn	Onexpected bacomes were satisfactority explained.			
Additional Comments:	This form re	presents growth outcomes associated with	Dry Weight	reported for Plantago shoot and roots within Figure 3 on page 6/10.			
<u> </u>		•					
Overall Qualit	ty Detern	nination	High				

Study Citation:	Ma, T., Teng Frontiers of l	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	tion: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	(0-96h)			
Exposure Route, Media. Path:	Terrestrial; S	oil; 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	/ascular Plants; Raphanus sativus; Embryo					
Health Outcome:	Reproductive	e/Teratogenic					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	Ce Matria 1.	Test Substance Identity	Low				
	Metric 2:	Test Substance Source	Low	Chemical was identified by name only.			
	mente 2.	rest Substance Source	LUW	performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%			
Domain 2: Test Desi							
Domanii 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 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 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.			
	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	C C	response.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds 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	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:	A deguacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of creation			
			nigii	health.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.			
		Conti	nued on nex	at page			

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May 2025

Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 2915866 Table: 1 of 3

continued from previous page							
Study Citation:	Ma, T., Teng	g, Y., Christie, P., Luo, Y. (2015). Phytotox	cicity in seve	en higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate.			
	Frontiers of	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; <i>Raphanus sativus</i> ; Embryo	•				
Health Outcome:	Reproductiv	e/Teratogenic					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.			
	Assessment						
Domain 6: Confounding	r / Variable Co	ntrol					
Domain 0. Comountaing	Matric 10	Confounding Variables in Test	High	There were no reported differences among the study groups in anyironmental conditions			
	Methe 19.	Design and Presedures	Ingn	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			
	Weute 20.	Sucomes chickated to Exposure	Wiedium	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:	This form re	presents the germination rate results presen	ited in Table	1 for Raphanus sativus with DBP exposure.			
Overall Qualit	ty Detern	nination	High				

PUBLIC RELEASE DRAFT

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>Raphanus sativus</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosynthesis Dibutyl phthalate (DBP) 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						
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 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	8	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.		

Continued on next page ...

PUBLIC RELEASE DRAFT May 2025

Environmental Hazard Evaluation

HERO ID: 2915866 Table: 2 of 3

Ma, T., Teng	Y., Christie, P., Luo, Y. (2015). Phytotox	, .			
TTOILUCIS OF I	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 Dura	tion: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	(0-96h)		
Terrestrial; S	oil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Vegetation; V	Vascular Plants; Raphanus sativus; Embryo				
Mechanistic-	Biomarkers (exposure and effect)-Photosy	nthesis			
Dibutyl phth	alate (DBP)				
2915866					
	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.		
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.		
tion 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.		
This is for pi contents."	gment content."The results indicate that lo	nger periods	of cultivation of the test plants may make it easier to interpret the changes in pigment		
	Terrestrial; S Vegetation; V Mechanistic- Dibutyl phth 2915866 / Variable Con Metric 19: Metric 20: ttion and Anal Metric 21: Metric 22: Metric 23: This is for pi contents."	Terrestrial; Soil; Not determined by study authors (i.e., Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosyn Dibutyl phthalate (DBP) 2915866 Metric / Variable Control Metric 19: Confounding Variables in Test Design and Procedures Metric 20: Outcomes Unrelated to Exposure ttion and Analysis Metric 21: Statistical Methods Metric 22: Reporting of Data Metric 23: Explanation of Unexpected Outcomes This is for pigment content."The results indicate that lo contents."	Terrestrial; Soil; Not determined by study authors (i.e., chemical of Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo Mechanistic-Biomarkers (exposure and effect)-Photosynthesis Dibutyl phthalate (DBP) 2915866 <u>Metric 19</u> : Confounding Variables in Test High Design and Procedures Metric 20: Outcomes Unrelated to Exposure Medium ttion and Analysis Metric 21: Statistical Methods Low Metric 22: Reporting of Data High Metric 23: Explanation of Unexpected Outcomes High This is for pigment content."The results indicate that longer periods contents."		

Overall Quality Determination

High

Dibutyl Phthalate

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: Health Outcome: Chemical: HERO ID:	Vegetation; V Development Dibutyl phth 2915866	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 2915866						
Domain		Metric	Rating	Comments				
Domain 1: Test Substand	ce							
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low Low	Chemical was identified by name only. 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								
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 7:	Experimental System/Test Media Preparation	Hıgh	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/ 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 Organisi	n							
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:	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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Dibutyl 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; Raphanus sativus; Embryc)			
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
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	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:	Virgin, H. I.	, A-M, Holst, Morner, J. (1981). Effect of	f di-n-butylphthalate on the ca	rotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-						
Duration:	163. Overall Dura	ation: Not-reported: Exposure Duration: N	lot-reported							
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e.,	chemical of interest in exposu	re water, but unable to determine exact uptake route)						
Media, Path:										
Taxa, Species, Age:	Vegetation; Mortality	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Cherry Belle; Not Applicable (e.g., fungi or algae studies) or Not Reported Mortality								
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)								
HERO ID:	1333234	1333234								
Domain		Metric	Rating	Comments						
Domain 1: Test Substan	ce		-							
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only						
	Metric 2: Matria 2:	Test Substance Source	Hign Madium	The test substance identity was analytically verified by the performing laboratory.						
	Meure 5.	Test Substance Furity	Medium							
Domain 2: Test Design										
	Metric 4:	Negative Controls	Low	Untreated plants were used as controls but few details were provided						
	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									
1	Metric 7:	Experimental System/Test Media Preparation	Medium	Several methods of obtaining vapors was reported but effects were not attributed to a particular method						
	Metric 8:	Consistency of Exposure	Low	Difficult to determine as durations and concentrations were not clearly reported						
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported						
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days but study length was not clearly reported						
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the number of exposure groups, the range seems to be						
		Spacing of Exposure Levels		10-150 ng/L but unclear if this is one treatment that varied over time or if there were multiple treatment levels						
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via vapors						
Domain 4. Test Organis	m									
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, age or stage at test initiation was not						
	Metric 14:	Acclimatization and Pretreatment	Low	clear. The study did not report whether test organisms were acclimatized						
		Conditions								
	Metric 15:	Number of Organisms and	Low	Numbers and replicates were not reported						
		Replicates per Group								
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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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333234 Table: 1 of 3

		cont	inued from previous	s page			
Study Citation:	Virgin, H. I.,	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-					
Dermetterne	163.	163. Overall Duration, Net reported, Europeuro Duration, Net reported					
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported Terrestrial: Air: Net determined by study outbors (i.e., shemical of interest in exposure water, but upoble to determine exect uptake route)						
Exposure Route,	Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
viedia, Pain:	X 7 4 4 X						
laxa, Species, Age:	Vegetation; V	Vascular Plants; Raphanus sativus; Cherry Belle	e; Not Applicable (e.g	g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality						
Unemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1333234						
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 & Confounding	Variable Cau	ataal					
Domain 6: Confounding	/ Variable Cor	IIIOI Confounding Variables in Test	Low				
	Metric 19:	Confounding variables in Test	LOW	conditions			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups			
	Wiethe 20.	Outcomes Oniciated to Exposure	Wiedrum	There was no information in the study to suggest unreferences among groups			
Domain 7: Data Present	ation and Anal	vsis					
Domain 7. Data i resent		,					
Domain 7. Data i resent	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, information was not quantified			
	Metric 21: Metric 22:	Statistical Methods Reporting of Data	N/A Low	Statistical analysis was not possible, information was not quantified There was brief mention of plant mortality in the results section text. No data was shown.			
	Metric 21: Metric 22: Metric 23:	Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	N/A Low Low	Statistical analysis was not possible, information was not quantified There was brief mention of plant mortality in the results section text. No data was shown. The study did not report any measures of variability			

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-					
Duration	163. Overall Dur	163. Overall Duration: Not-reported: Exposure Duration: Not-reported					
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e., c	hemical of interest in exposi-	re water, but unable to determine exact uptake route)			
Media. Path:	Terrestriar, 1		nemieur of interest in exposi				
Taxa, Species, Age:	Vegetation:	Vegetation: Vascular Plants; <i>Raphanus sativus</i> ; Cherry Belle; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic	Mechanistic-Photosynthesis					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)					
HERO ID:	1333234						
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	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	Medium	Pro analysis quality was reported.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	Low	Untreated plants were used as controls, but few details were provided.			
	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	Several methods of obtaining vapors were reported, but effects were not attributed to a particular method.			
	Metric 8:	Consistency of Exposure	Low	The consistency of exposure was difficult to determine as durations and concentrations were not clearly reported.			
	Metric 9:	Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported.			
	Metric 10:	Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days but study length was not clearly reported.			
	Metric 11:	Number of Exposure Groups/	Uninformative	No information is provided on the number of exposure groups, the range seems to be			
		Spacing of Exposure Levels		10-150 ng/L, but it's unclear if this is one treatment that varied over time or if there were multiple treatment levels.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via vapors.			
Domain 4 [.] Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, and age or stage at test initiation was not clear.			
	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	Numbers of organisms and replicates were not reported.			
		· · · · · · · · · · · · · · · · · · ·					
Domain 5: Outcome As	sessment		Ŧ				
	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 Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were con- fusing.			
			Continued on next page				

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Dibutyl Phthalate

Environmental Hazard Evaluation

HERO ID: 1333234 Table: 2 of 3

continued from previous page					
Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-			
Duration:	163. Overall Dura	ation: Not-reported: Exposure Duration: Not-r	reported		
Exposure Route,	Terrestrial; A	Air; Not determined by study authors (i.e., che	mical of interest in exp	osure water, but unable to determine exact uptake route)	
Media, Path:		• • •			
Taxa, Species, Age:	Vegetation; Y	Vascular Plants; Raphanus sativus; Cherry Bel	lle; Not Applicable (e.g	g., fungi or algae studies) or Not Reported	
Health Outcome:	Mechanistic	-Photosynthesis			
Chemical:	Dibutyl phth	alate (DBP)			
HERO ID:	1333234				
Domain		Metric	Rating	Comments	
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	
	Matria 20:	Design and Procedures Outcomes Unrelated to Exposure	Madium	Conditions.	
	Metric 20.	Outcomes Onierated 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	N/A	Statistical analysis was not possible, and information was not quantified.	
	Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes.	
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.	
Additional Comments:	The test syst	em was closed, and light was a variable.			

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Virgin, H. I.,	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-				
Duration:	163. Overall Dura	163. Overall Duration: Not-reported: Exposure Duration: Not-reported				
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e.	, chemical of interest in exposu	re water, but unable to determine exact uptake route)		
Media. Path:	10110001101,11		, enemear of meress in enpose			
Taxa, Species, Age:	Vegetation: V	Vascular Plants: Raphanus sativus: Cherry	y Belle: Not Applicable (e.g., fu	ungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-	Cytotoxicity-Photosynthesis	, ,			
Chemical:	Dibutyl phtha	alate (DBP)				
HERO ID:	1333234	1333234				
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	High	The test substance identity was analytically verified by the performing laboratory.		
	Metric 3:	Test Substance Purity	Medium	pro analysis quality was reported		
Domain 2: Test Design						
,	Metric 4:	Negative Controls	Low	Untreated plants were used as controls but few details were provided		
	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	Medium	Several methods of obtaining vapors was reported but effects were not attributed to a		
		Preparation	Ŧ	particular method		
	Metric 8:	Consistency of Exposure	Low	Difficult to determine as durations and concentrations were not clearly reported		
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured but not clearly reported		
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures seemed to be around 4-7 days but study length was not clearly reported		
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups, the range seems to be 10-150 ng/L but unclear if this is one treatment that varied over time or if there were multiple treatment levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	exposure was via vapors		
Domain 4: Test Organis	m		Ŧ			
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported, age or stage at test initiation was not clear.		
	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	Numbers and replicates were 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 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 con-		
		Assessment		fusing		
			Continued on next page			

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Environmental Hazard Evaluation

Dibutyl Phthalate

HERO ID: 1333234 Table: 3 of 3

		0	ontinued from previous	page		
Study Citation:	Virgin, H. I.	Virgin, H. I., A-M, Holst, Morner, J. (1981). Effect of di-n-butylphthalate on the carotenoid synthesis in green plants. Physiologia Plantarum 53(2):158-				
Duration:	163. Overall Dura	163. Overall Duration: Not-reported: Exposure Duration: Not-reported				
Exposure Route,	Terrestrial; A	Terrestrial; Air; 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; Cherry B	elle; Not Applicable (e.g	g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic	-Cytotoxicity-Photosynthesis				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1333234					
Domain		Metric	Rating	Comments		
Domain 6: Confounding	g / Variable Con 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		
	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	ysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not possible, information was not quantified		
	Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability		
Additional Comments:	open system	, light was a variable				
Overall Quali	ty Detern	nination	Uninformativ	e		

Study Citation:	Lã,Kke, H.,	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and					
Duration: Exposure Route, Media. Path:	field experin Overall Dur Terrestrial; I	nents. Environmental Pollution Series A: Eco ation: 0 - 4 days (0-96h); Exposure Duration: N/A (e.g., injection); Not determined by study	ological and Biological 0 - 4 days (0-96h) y authors (i.e., chemical	of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age: Health Outcome:	Vegetation; Skin & Con	Vegetation; Vascular Plants; <i>Sinapis Alba</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Skin & Connective Tissue					
Chemical: HERO ID:	Dibutyl phth 9430481	Dibutyl phthalate (DBP) 9430481					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce	— • • • •					
	Metric 1:	Test Substance Identity	Low	Chemicals were identified as di-(2-ethyl hexyl)-phthalate (DEHP) and di-(n-butyl)- phthalate (DBP). No CASRN was provided.			
	Metric 2:	Test Substance Source	High	DBP was sourced from Fluka, Switzerland, and DEHP was sourced from Scandiflex, Ltd., Denmark. Analytical verification was conducted.			
	Metric 3:	Test Substance Purity	High	DPB purity was >99.5% and DEHP purity was >99%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative controls included solvent-treated (acetone (5%) + water (95%) + Tween 20 (0.1%)) plots.			
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups is shown in Table 2 as no injury observed.			
	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	The plants were treated with a 2.5 m two-person operated pneumatic sprayer mounted with five flat spray nozzles with constant pressure applied to give a known amount of liquid per unit time at 35 mL per square meter. Chemicals were prepared in solvent as			
			_	described in the laboratory experiments.			
	Metric 8:	Consistency of Exposure	Low	It is unclear what plots were treated at which times.			
	Metric 9:	Administration Measurement of Test Substance	Medium	The test concentrations were measured in the leaves in Sinapis at 1 and 3-hr post- application			
	Metric 10:	Exposure Duration and Frequency	Medium	It was applied one time with a solvent. It was reported that a thunderstorm 20 hours after treatment may have ended the exposure.			
	Metric 11:	Number of Exposure Groups/	Medium	Exposure concentrations are shown in Table 2.			
	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. Application concentrations were presented in terms of			
				mL per square meter. A solvent was used, so this likely enhanced the solubility of the test material.			
Domain 4: Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The test species were Brassica and Sinapis. No source was provided for the seeds.			
		Co	ontinued on next page				

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Environmental Hazard Evaluation

HERO ID: 9430481 Table: 1 of 1

continued from previous page							
Study Citation:	Lã,Kke, H., field experin	Lã, Kke, H., Rasmussen, L. (1983). Phytotoxicological effects of Di-(2-ethyl hexyl)-phthalate and Di-n-butyl-phthalate on higher plants in laboratory and field experiments. Environmental Pollution Series A: Ecological and Biological 32(3):179-199.					
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; N	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:	Vegetation;	Vascular Plants; <i>Sinapis Alba</i> ; Not Applicable	(e.g., fungi or algae stu	dies) or Not Reported			
Health Outcome:	Skin & Conr	Skin & Connective Tissue					
Unemical:		lalate (DBP)					
	9450461						
Domain		Metric	Rating	Comments			
	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	The number of test organisms and/or replicates was not reported.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and/or environmental conditions and/or food, water, and 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	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 (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.			
	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	Uninformative	No statistical analysis was conducted.			
	Metric 22:	Reporting of Data	Low	There was minimal reporting of data in the text of the study (data for most treatment groups was not described). Data in Table 2 was represented as +/			
	Metric 23:	Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred. Authors reported thunderstorms approximately one day after spraying both species.			
Additional Comments:	This form ap of chemical Table 2 repo	pplies to both Sinapis and Brassica. DEHP, D on plant measured at 1 and 3 hours post spray rts injury as a +/- (not quantified).	BP, or a mixture was sp ying. Injury described a	rayed onto plants in plots at doses described in Table 2. Concentrations s chlorotic spots. Results in the text were not well described/quantified.			

Overall Quality Determination

Uninformative

Study Citation:	Løkke, H., H (DEHP) by J	Løkke, H., Bro-Rasmussen, F. (1981). Studies of mobility of di-iso-butyl phthalate (DiBP), di-N-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10(11-12):1223-1236.						
Exposure Route.	Terrestrial: V	Terrestrial: Water: Dermal (topical application)						
Media, Path:	Terrestriar,	Vegetation; Vascular Plants; <i>Sinapsis alba</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported ADME (biotransformation)						
Taxa, Species, Age:	Vegetation;							
Health Outcome:	ADME (bio							
Chemical:	Dibutyl phth	nalate (DBP)						
HERO ID:	680337							
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce	— • • • •	-					
	Metric 1:	Test Substance Identity	Low	Identified by nomenclature (Di-n-butyl phthalic acid ester). No other identifying infor- mation provided.				
	Metric 2:	Test Substance Source	High	Fluke AG, CH-9470 Buchs, Switzerland				
	Metric 3:	Test Substance Purity	High	>99.5% purity.				
Domain 2: Test Design								
-	Metric 4:	Negative Controls	High	Negative & positive (carrier) controls were present.				
	Metric 5:	Negative Control Response	Low	Biological response of negative controls 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	High	Experimental system & test media were reported in detail.				
		Preparation	Ŧ					
	Metric 8:	Administration	Low	Exposures were administered consistently, but spraying efficiency was "estimated to be 65-75% for Sinapis alba and Lapsana communis, and 40-50% for Achillea millefol- lum" with no further information given. Therefore, exposure was inconsistent among & within the study groups.				
	Metric 9:	Measurement of Test Substance Concentration	Low	Given doses for the DBP experiment (1.5, 0.25, and 0.05 ug/ cm2 respectively of the whole leaf area) were not analytically verified & due to the spray efficiency concerns noted in Metric 8 cannot be expected to be similar to nominal concentrations.				
	Metric 10:	Exposure Duration and Frequency	High	Single exposure was performed with monitoring for 15 days.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Response was only found at the highest dose and there was a closely-spaced dose below that, indicating that lower doses are low enough to establish a NOEC/LOEC.				
	Metric 12:	Testing at or Below Solubility Limit	High	No biological responses found in the solvent controls.				
Domain 4: Test Organis	m Metric 12:	Test Organism Characteristics	Low	Source of test species was not reported				
	Metric 14	Acclimatization and Pretreatment	LOW	Source or rest species was not reported. Plants were not acclimatized to the elaborate climate chamber before the experiment				
	MCUIC 17.	Conditions	Low	began, and were instead reared outside it and placed inside immediately upon exposure. This chamber may have had an impact on the results.				
	Metric 15:	Number of Organisms and Replicates per Group	Low	8 plants were used per group, with no replication.				

Continued on next page ...

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Dibutyl Phthalate

		co	ntinued from previous	page				
Study Citation:	Løkke, H., F (DEHP) by p	Løkke, H., Bro-Rasmussen, F. (1981). Studies of mobility of di-iso-butyl phthalate (DiBP), di-N-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10(11-12):1223-1236.						
Duration:	Overall Dura	Terrestrial: Water: Dermal (tonical application)						
Exposure Koule, Modio Dothe	Terrestriar; v	Terrestrial; Water; Dermal (topical application)						
Teve Species Ages	Vagatation	Vescular Dients: Singnois alba: Not Applicabl	a (a g fungi ar algaa sti	idias) or Not Deported				
Haalth Outcomer	ADME (biot	vascular Flams, <i>Sinupsis alba</i> , Not Applicable	e (e.g., fungi of algae su	dules) of Not Reported				
Chamicali	Dibutyl phth	nalistormation)						
HERO ID:	680337	larate (DDF)						
Domain		Metric	Rating	Comments				
Domain 5: Outcome As	sessment							
	Metric 16:	Adequacy of Test Conditions	High	Organism housing characteristics were described in detail & were appropriate for the rearing of plants.				
	Metric 17:	Outcome Assessment Methodology	Medium	The assessment methodology was not described. It was simply stated that "chlorosis developed in the new leaves".				
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding outcome assessment were not reported.				
Domain 6: Confounding	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Uninformative	The DBP-treated plants were housed in the same sealed chamber as plants treated with DEHP and DiBP, virtually guaranteeing cross-contamination. The controls were separated from the other plants.				
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not performed.				
	Metric 22:	Reporting of Data	Low	Data for control groups was not described in the text (was there any chlorosis in non- treated groups?)				
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes.				
Additional Comments:	The only stu where DBP, endpoint. T	The only study reported in this paper that was PECO-relevant is the exposure to DBP in Sinapis alba – the other experiments detailed are mixture studies where DBP, DiBP, and DEHP were applied. The DBP-alone exposure DBP residues in plants as a function of time, and observed chlorosis as the apical endpoint. This study had several methodological flaws, including housing DBP-treated plants in the same sealed chamber as plants treated with other						

Overall Quality Determination

Uninformative

phthalates, virtually guaranteeing cross-contamination. This form is for the ADME outcome.

Study Citation: Duration: Exposure Route, Media Path:	Løkke, H., Bro-Rasmussen, F. (1981). Studies of mobility of di-iso-butyl phthalate (DiBP), di-N-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10(11-12):1223-1236. Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h) Terrestrial; Water; Dermal (topical application)				
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; ADME (bio Dibutyl phth 680337	Vascular Plants; <i>Sinapsis alba</i> ; Not Applicabl transformation) aalate (DBP)	e (e.g., fungi or algae s	studies) or Not Reported	
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	ce Metric 1:	Test Substance Identity	Low	The test substance was identified by nomenclature (Di-n-butyl phthalic acid ester). No other identifying information was provided.	
	Metric 2:	Test Substance Source	High	The source was Fluke AG, CH-9470 Buchs, Switzerland.	
	Metric 3:	Test Substance Purity	High	The purity was reported as >99.5%.	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Negative & positive (carrier) controls were present.	
	Metric 5:	Negative Control Response	Low	Biological response of negative controls was not reported.	
	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 & test media were reported in detail.	
	Metric 8:	Consistency of Exposure Administration	Low	Exposures were administered consistently, but spraying efficiency was "estimated to be 65-75% for Sinapis alba and Lapsana communis, and 40-50% for Achillea millefol- lum" with no further information given. Therefore, exposure was inconsistent among & within the study groups.	
	Metric 9:	Measurement of Test Substance Concentration	Low	Given doses for the DBP experiment (1.5, 0.25, and 0.05 ug/ cm2 respectively of the whole leaf area) were not analytically verified & due to the spray efficiency concerns noted in Metric 8, they cannot be expected to be similar to nominal concentrations.	
	Metric 10:	Exposure Duration and Frequency	High	Single exposure was performed with monitoring for 15 days.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Response was only found at the highest dose and there was a closely-spaced dose below that, indicating that lower doses are low enough to establish a NOEC/LOEC.	
	Metric 12:	Testing at or Below Solubility Limit	High	No biological responses were found in the solvent controls.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	Low	Source of test species was not reported.	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Plants were not acclimatized to the elaborate climate chamber before the experiment began, and were instead reared outside it and placed inside immediately upon exposure. This chamber may have had an impact on the results.	
	Metric 15:	Number of Organisms and Replicates per Group	Low	Eight plants were used per group, with no replication.	

Domain 5: Outcome Assessment

Continued on next page ...

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HERO ID: 680337 Table: 2 of 2

		col	ntinued from previous	page			
Study Citation:	Løkke, H., F (DEHP) by 1	Løkke, H., Bro-Rasmussen, F. (1981). Studies of mobility of di-iso-butyl phthalate (DiBP), di-N-butyl phthalate (DBP), and di-(2-ethyl hexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10(11-12):1223-1236.					
Duration:	Overall Dura	verall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h)					
Exposure Route,	Terrestrial; V	Terrestrial; Water; Dermal (topical application)					
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vegetation; Vascular Plants; Sinapsis alba; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	transformation)					
Chemical: HERO ID:	Dibutyl phth 680337	nalate (DBP)					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Organism housing characteristics were described in detail & were appropriate for the rearing of plants.			
	Metric 17:	Outcome Assessment Methodology	Medium	The assessment methodology was not described. It was simply stated that "chlorosis developed in the new leaves."			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding outcome assessment were not reported.			
Domain 6: Confounding	y / Variable Co	ntrol					
Bonnam of Contouring	Metric 19:	Confounding Variables in Test	Uninformative	The DBP-treated plants were housed in the same sealed chamber as plants treated with			
		Design and Procedures		DEHP and DiBP, virtually guaranteeing cross-contamination. The controls were sepa- rated from the other plants.			
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no outcomes unrelated to exposure.			
Domain 7: Data Present	ation and Anal	lysis					
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not performed.			
	Metric 22:	Reporting of Data	Low	Data for control groups was not described in the text (it is unknown if there was any chlorosis in the non-treated groups).			
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexplained outcomes.			
Additional Comments:	The only stu where DBP, apical endpo other phthala	dy reported in this paper that was PECO-rele DiBP, and DEHP were applied. The DBP-al pint. This study had several methodological ates, virtually guaranteeing cross-contamination	vant is the exposure to I lone exposure used DBI flaws, including housing on. This form is for chlo	DBP in Sinapis alba – the other experiments detailed are mixture studies P residues in plants as a function of time, and observed chlorosis as the g DBP-treated plants in the same sealed chamber as plants treated with prosis.			

Overall Quality Determination

Dibutyl Phthalate

Uninformative

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Study Citation:	Kannan, S. Nutrition 9(1	(1986). Effects of dibutyl phthalate and ph	thalic-acid on chlorosis re	ecovery in iron-deficiency stressed sorghum cultivars. Journal of Plant		
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 -	21 days			
Exposure Route,	Terrestrial; S	Soil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation: Vascular Plants; Sorghum bicolor; cv. CSH-5; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biotransformation)					
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)				
HERO ID:	5433174					
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 or grade of test substance was not reported.		
Domain 2: Test Design						
Domain 21 Teor Deorgi	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group		
	Metric 5:	Negative Control Response	Low	A picture of the control response was all that 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 no details on the measures taken to appropriately prepare test con- centrations		
	Metric 8:	Consistency of Exposure	Low	Only general methods of exposure administration were reported so assessment was		
		Administration		difficult to determine		
	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 suitable for the study type		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration tested		
		Spacing of Exposure Levels	27/4			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil		
Domain 4: Test Organis	m					
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds 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 or replicates was not reported.		
		Replicates per Group				
Domain 5: Outcome As	sessment		T			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate		
	Metric 17:	Outcome Assessment Methodology	Low	Root growth was not quantitatively reported		
		C	Continued on next page .			

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Dibutyl Phthalate

HERO ID: 5433174 Table: 1 of 4

		CO	ntinued from previous	page		
Study Citation:	Kannan, S. (1986). Effects of dibutyl phthalate and phthalic-acid on chlorosis recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant					
	Nutrition 9(12):1543-1551.					
Duration:	Overall Dura	ation: 11 - 21 days; Exposure Duration: 11 - 2	21 days			
Exposure Route,	Ierrestrial; Soii; Root uptake					
Media, Path:						
Taxa, Species, Age:	Vegetation; Vascular Plants; Sorghum bicolor; cv. CSH-5; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biot	ransformation)				
Chemical:	Dibutyl phthalate (DBP)					
HERO ID:	5433174					
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	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	veie				
Domain 7. Data i resent	Metric 21.	Statistical Methods	Uninformative	Statistical analysis was not conducted		
	Metric 22:	Reporting of Data	Uninformative	Only figures were presented		
	Metric 22.	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability		
	Methe 25.	Explanation of Onexpected Outcomes	LUW	The study did not report any measures of variability		
Additional Comments:	recovery from	m chlorosis was noted but not quantified				
Overall Qualit	tv Detern	nination	Uninformative	2		

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Study Citation:	Kannan, S.	(1986). Effects of dibutyl phthalate and phth	nalic-acid on chlorosis	recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant			
Desition	Nutrition 9(12):1543-1551.	21.1				
Duration:	Overall Dur	ation: 11 - 21 days; Exposure Duration: 11 - 2	21 days				
Exposure Koule, Modia Dath:	Terrestrial; S	Terrestriar, Son, Root uptake					
Toyo Species Age:	Vegetation: Vascular Plants: Sorghum bicolor: cv. CS-3541: Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome	Developmer	vasculai Flains, <i>Sorgnum Dicolor</i> , ev. CS-354	ri, Not Applicable (e.g	, fungi of argae studies) of Not Reported			
Chemical:	Dibutyl phth	valate (DBP)					
HERO ID:	5433174						
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	ice						
	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 the test substance was not reported.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 5:	Negative Control Response	Low	A picture of the control response was all that was 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	Low	The study provided no details on the measures taken to appropriately prepare test con-			
		Preparation		centrations.			
	Metric 8:	Consistency of Exposure	Low	Only general methods of exposure administration were reported, so assessment was			
	Matria O.	Administration	Ι	difficult to determine.			
	Metric 9:	Concentration	LOW	Exposure concentrations were not measured.			
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and suitable for the study type.			
	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	Exposure was via soil.			
Domain 4: Test Organis	sm						
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds 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	The number of test organisms or replicates was not reported.			
		Replicates per Group					
Domain 5: Outcome As	ssessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Metric 17:	Outcome Assessment Methodology	Low	Root growth was not quantitatively reported.			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported.			
		Co	ontinued on next page				

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Dibutyl Phthalate

		coi	ntinued from previous p	page			
Study Citation:	Kannan, S. (1986). Effects of dibutyl phthalate and phthalic-acid on chlorosis recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant						
Duration	Nutrition 9(12):1543-1551. Overall Duration: 11 - 21 days: Exposure Duration: 11 - 21 days						
Exposure Route	Terrestrial: S	ail: Root untake	1 days				
Media Path	Terrestriar, 5	ion, Root uptake					
Toyo Spacios Ago:	Vagatation: V	Vascular Dante: Sarahum bicalar: av CS 354	1. Not Applicable (e.g.	funcior algae studies) or Not Peported			
Hoolth Outcomo:	Developmen	t/Growth	1, Not Applicable (e.g.,	fungi of argae studies) of Not Reported			
Chamicali	Developmen Dibutul phth	elete (DPD)					
	5/3317/	SA22174					
IIERO ID.	5455174						
Domain		Metric	Rating	Comments			
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 7: Data Present	ation and Anal	ysis					
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.			
	Metric 22:	Reporting of Data	Uninformative	Only figures were presented.			
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.			
Additional Comments:	Recovery fro	om chlorosis was noted but not quantified.					

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Kannan, S.	(1986). Effects of dibutyl phthalate and phth	nalic-acid on chlorosis	recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant			
Duration:	Nutrition 9(Overall Dur	utrition 9(12):1543-1551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route.	Terrestrial: S	Soil; Root uptake	21 auyo				
Media, Path:	· · · , ·	Vegetation; Vascular Plants; Sorghum bicolor; cv. CSH-5; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Taxa, Species, Age:	Vegetation;						
Health Outcome:	Developmer	nt/Growth					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	5433174						
Domain		Metric	Rating	Comments			
Domain 1: Test Substar	ice						
	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 or grade of test substance was 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	Low	A picture of the control response was all that 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	Low	The study provided no details on the measures taken to appropriately prepare test con-			
		Preparation		centrations			
	Metric 8:	Consistency of Exposure	Low	Only general methods of exposure administration were reported so assessment was			
	Matria O.	Administration	Ι	difficult to determine			
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured			
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and suitable for the study type			
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration tested			
		Spacing of Exposure Levels					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil			
Domain 4. Test Organis	m						
2 onium 1. rost organie	Metric 13.	Test Organism Characteristics	Low	The source of the test seeds was not reported			
	Metric 14	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized			
		Conditions	20				
	Metric 15:	Number of Organisms and	Low	The number of test organisms or replicates was not reported.			
		Replicates per Group					
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	Root growth was not quantitatively reported			
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not			
		Assessment		reported			
		Co	ontinued on next page				

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Dibutyl Phthalate

		coi	ntinued from previous	page		
Study Citation:	Kannan, S. (1986). Effects of dibutyl phthalate and phthalic-acid on chlorosis recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant					
	Nutrition 9(12):1543-1551.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; S	oil; Root uptake				
Media, Path:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Sorghum bicolor; cv. CSH-5;	Not Applicable (e.g., fu	ingi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5433174					
Domain		Metric	Rating	Comments		
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 7. Data Dragant	otion and Anal					
Domain 7: Data Present	Matria 21.	ysis	T T f			
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Uninformative	Only figures were presented		
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability		
Additional Comments:	recovery from	n chlorosis was noted but not quantified				

Overall Quality Determination

Uninformative

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HERO ID: 5433174 Table: 4 of 4

Study Citation:	Kannan, S.	(1986). Effects of dibutyl phthalate and phth	alic-acid on chlorosis	recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant		
Duration: Exposure Route, Media, Path:	Nutrition 9(Overall Dur Terrestrial; S	Nutrition 9(12):1545-1551. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days Terrestrial; Soil; Root uptake				
Taxa, Species, Age: Health Outcome:	Vegetation; Vascular Plants; Sorghum bicolor; cv. 2077-5; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth					
Chemical:	Dibutyl phth	nalate (DBP)				
HERO ID:	5433174					
Domain		Metric	Rating	Comments		
Domain 1: Test Substance	e					
	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 or grade of test substance was not reported.		
Domain 2: Test Design						
6	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group		
	Metric 5:	Negative Control Response	Low	A picture of the control response was all that was reported		
	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 no details on the measures taken to appropriately prepare test con- centrations		
	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:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and suitable for the study type		
	Metric 11:	Number of Exposure Groups/	N/A	Only one concentration tested		
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	Exposure was via soil		
Domain 4: Test Organism	n					
Domain 1. Tost Organish	Metric 13	Test Organism Characteristics	Low	The source of the test seeds 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 or replicates was not reported.		
		Replicates per Group				
Domain 5: Outcome Ass	essment					
Domain 5. Outcome Ass	Metric 16.	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate		
	Metric 17	Outcome Assessment Methodology	Low	Root growth was not quantitatively reported		
	Metric 18	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not		
	wieute 18:	Assessment	LUW	reported		
		<u> </u>	ntinued on post page			

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Dibutyl Phthalate

		coi	ntinued from previous	page		
Study Citation:	Kannan, S. (Kannan, S. (1986). Effects of dibutyl phthalate and phthalic-acid on chlorosis recovery in iron-deficiency stressed sorghum cultivars. Journal of Plant				
	Nutrition 9(12):1543-1551.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	Terrestrial; Soil; Root uptake					
Media, Path:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Sorghum bicolor; cv. 2077-5;	Not Applicable (e.g., fu	ingi or algae studies) or Not Reported		
Health Outcome:	Developmen	t/Growth				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	5433174					
Domain		Metric	Rating	Comments		
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 7: Data Present	ation and Anal	veic				
Domain 7. Data i resent	Motric 21.	Statistical Methods	Uninformative	Statistical analysis was not conducted		
	Metric 21.	Benerting of Data	Uninformative	Statistical analysis was not conducted.		
	Metric 22:	Reporting of Data	Uninformative	Only ngures were presented		
	Metric 23:	Explanation of Unexpected Outcomes	LOW	The study did not report any measures of variability		
Additional Comments:	recovery from	n chlorosis was noted but not quantified				

Overall Quality Determination

Uninformative

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Dueck, T. A	., Dijk, Van, C. J., David, F., Scholz, N., V	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant			
Duration	species. Che	mosphere $53(8):911-920$.	1 days				
Exposure Route.	Terrestrial: A	Terrestrial: Air: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	Terrestitui, T	in, i tot determined by study dutions (ne.,	chemical of I				
Taxa, Species, Age:	Vegetation; V	Vegetation; Vascular Plants; Trifolium repens; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	1302103						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		*** 1				
	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	Purity or 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, note that all controls had some DBP detected. Control group DBP concentrations are reported in Table 1.			
	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	Matria 7	Experimental System/Test Media	High				
	Metric 7:	Preparation	Fign	adeguate detail. The chambers were constructed of "hardened glass" and aluminum.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups based on timed sam- plings.			
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."			
	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	The number of exposure groups and spacing of exposure levels were suitable. Concen-			
		Spacing of Exposure Levels		tration gradient is reported as both actual and nominal within table 1.			
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.			
Domain 4. Test Organis	m						
	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.			
		Cont	inued on nex	ct page			
		Cont		LQ			

Environmental Hazard Evaluation

HERO ID: 1302103 Table: 1 of 2

		conti	nued from p	previous page			
Study Citation:	Dueck, T. A	., Dijk, Van, C. J., David, F., Scholz, N., V mosphere 53(8):911-920	/anwallegher	m, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant			
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	davs				
Exposure Route.	Terrestrial: A	Air: Not determined by study authors (i.e., o	chemical of i	interest in exposure water, but unable to determine exact uptake route)			
Media. Path:							
Taxa, Species, Age:	Vegetation; Vascular Plants; Trifolium repens; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	1302103						
Domain		Metric	Rating	Comments			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were four plants per treatment and no replicates reported. "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum num-			
Domain 5: Outcome As	sessment Metric 16: Metric 17: Metric 18:	Adequacy of Test Conditions Outcome Assessment Methodology Consistency of Outcome Assessment	High High Medium	Environmental conditions of test system were conducive to maintenance of organism health. The outcome assessment methodology reported the intended outcome of interest. Outcomes were assessed consistently across study groups but few details were reported.			
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	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:	This form re	presents growth outcomes associated with	Dry Weight	reported for Trifolium shoot and roots within Figure 3 on page 6/10.			
Overall Qualit	ty Detern	nination	High				

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HERO ID: 1302103 Table: 2 of 2

Study Citation:	Dueck, T. A	., Dijk, Van, C. J., David, F., Scholz, N.,	Vanwallegher	n, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant
	species. Che	emosphere 53(8):911-920.		
Duration:	Overall Dur	ation: > 21 days; Exposure Duration: > 2	1 days	
Exposure Route,	Terrestrial;	Air; Not determined by study authors (i.e.,	chemical of i	nterest in exposure water, but unable to determine exact uptake route)
Media, Path:				
Taxa, Species, Age:	Vegetation;	Vascular Plants; Trifolium repens; Not App	plicable (e.g.,	fungi or algae studies) or Not Reported
Health Outcome:	ADME (bio	transformation)		
Chemical:	Dibutyl phth	nalate (DBP)		
HERO ID:	1302103			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	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	Low	Purity or grade of test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group, note that
				Table 1
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for
		regative control response		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	High	The experimental system and methods for preparation of test media were described in
		Preparation		adequate detail. The chambers were constructed of "hardened glass" and aluminum.
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups based on timed sam-
		Administration		plings.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies. Samples were taken frequently: "Actual DBP concentrations were measured by taking air samples from each chamber using an automated gas sampling device (GS-1 gas sampler,Gerstel,M €ulheim, Germany). Sampling was performed with Tenax TA tubes (2,6-diphenylphenylene oxide polymer) at 250ml min1 for 20 min. During the fumigation experiment, each chamber was sampled in duplicate on a weekly basis during the complete exposure period (76 days)."
	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	Medium	The number of exposure groups and spacing of exposure levels were suitable. Concen- tration gradient is reported as both actual and nominal within table 1.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation in a continually renewed chamber.
Domain 4: Test Organisi	m			
	Metric 13:	Test Organism Characteristics	Low	The crop and herbaceous species originated from germinated seeds, but source was not explicitly reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.
		Cont	inued on nex	ct page
		001		

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Environmental Hazard Evaluation

HERO ID: 1302103 Table: 2 of 2

		conti	nued from p	previous page		
Study Citation:	Dueck, T. A	., Dijk, Van, C. J., David, F., Scholz, N., V	/anwallegher	m, F. (2003). Chronic effects of vapour phase di-n-butyl phthalate (DBP) on six plant		
Duration:	Overall Dura	ation: > 21 days: Exposure Duration: > 21	days			
Exposure Route.	Terrestrial; Air; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media. Path:	Terrestrial, Thi, Not determined by study dualors (i.e., enomical of interest in exposule which, but anable to determine exact uplate route)					
Taxa. Species. Age:	Vegetation: Vascular Plants: Trifolium repens: Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	ADME (biotransformation)					
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	1302103					
Domain		Metric	Rating	Comments		
	Metric 15:	Number of Organisms and Replicates per Group	Low	Four plants per treatment and no replicates reported, "Three to four weeks after sowing, when the first mature leaves appeared, four uniform individuals per species including the tree species were placed in each fumigation chamber (the maximum number physically possible) for exposure to DBP.		
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	Medium	Outcomes were assessed consistently across study groups but few details were reported.		
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	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 in Table 2.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.		
Additional Comments:	This form is	for ADME (biotransformation) assessmen	t of DBP cor	ncentration in leaf tissue.		
Overall Qualit	ty Detern	nination	High			

Study Citation:	Ma, T., Teng	g, Y., Christie, P., Luo, Y. (2015). Phytotox	kicity in seve	n higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate
Destination	Frontiers of	Environmental Science & Engineering 9(2)):259-268.	(0.0(1))
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	(0-96h)
Exposure Koule, Modia Dath	Terrestrial; S	soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)
Tava Spacias Agas	Vagatation	Vaccular Blanta: Tritiaum gastinum: Embru		
Haalth Outcomer	Perroductiv	vasculai Flants, <i>Trucum destivum</i> , Eliibiyo	5	
Chamical:	Dibutul phth	e/ reratogenic		
HERO ID.	2015866	lalate (DBF)		
IIERO ID.	2913800			
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 1.	Negative Controls	High	Study authors reported using an appropriate concurrent pagative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and ade
	Metric 5.	Negative Control Response	-	quate.
	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	High	The experimental system and methods for preparation of test media were described in
	medite 7.	Preparation	mgn	adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
		Administration	6	
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Matria 10.	Concentration	11:-1-	
	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
		Spacing of Exposure Levels		response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test Organis	m			
Domain +. 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
	mente 14.	Conditions	mgn	ran 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.

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Environmental Hazard Evaluation

HERO ID: 2915866 Table: 1 of 3

		····contin	nucu nom p	herious 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; 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:	Reproductive	e/Teratogenic				
Chemical:	Dibutyl phthalate (DBP)					
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	High	Outcomes were assessed consistently across study groups.		
		Assessment				
Domain 6: Confounding	/ 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 Presenta	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.		
	This form represents the germination rate results presented in Table 1 for Triticum aestivum with DBP exposure.					

Dibutyl Phthalate

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: Health Outcome: Chemical: HERO ID:	Vegetation; V Development Dibutyl phtha 2915866	/ascular Plants; <i>Triticum aestivum</i> ; Embryo t/Growth alate (DBP)	0			
Domain		Metric	Rating	Comments		
Domain 1: Test Substand	ce					
	Metric 1: Metric 2:	Test Substance Identity Test Substance Source	Low Low	Chemical was identified by name only. 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						
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 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/ 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 Organisi	n					
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:	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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Dibutyl Phthalate

		conti	nued from p	revious page			
Study Citation:	Ma, T., Teng	fa, 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	rontiers 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:	Dibutyl phth	alate (DBP)					
HERO ID:	2915866						
Domain		Metric	Rating	Comments			
Domain 6: Confounding	g / variable Co		TT' 1				
	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			
	Wieurie 20.	Outcomes Onielated to Exposure	Wedium	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 I However, the in the evalua	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on thegermination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growthand biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of thephytotoxicity of PAE compounds."					

Overall Quality Determination

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 Dibutyl phthalate (DBP) 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					
	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	27/4	response.	
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds were 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:	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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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	ation: 0 - 4 days (0-96h); Exposure Duratio	n: 0 - 4 days	s (0-96h)		
Exposure Route, Modia Bathy	Terrestrial; S	Soil; Not determined by study authors (i.e.,	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Tava Species Age	Vegetation	Vascular Plants: Triticum aestivum: Embryo	`			
Health Outcome	Mechanistic	-Biomarkers (exposure and effect) Photosy	nthesis			
Chamical:	Dibutyl phth	valate (DRP)	nuicsis			
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.		
	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 were adequate to determine values for the endpoint.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	This is for p contents."	igment content."The results indicate that lo	nger periods	s of cultivation of the test plants may make it easier to interpret the changes in pigment		
	contents."	• ,•				

Overall Quality Determination

High

Dibutyl Phthalate

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

Study Citation:	Gao, M., Guo, Z., Dong, Y., Song, Z. (2019). Effects of di-n-butyl phthalate on photosynthetic performance and oxidative damage in different growth						
Duration: Exposure Route, Modia, Path:	stages of wh Overall Dura Terrestrial; S	Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake					
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Developmen Dibutyl phth 5495646	Vegetation; Vascular Plants; <i>Triticum aestivum L.</i> ; Embryo Development/Growth Dibutyl phthalate (DBP) 5495646					
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.			
	Metric 2:	Test Substance Source	Low	The source was reported to be J & K Scientific Ltd. in Beijing, China. However, it was not reported if the DBP was analytically verified.			
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.			
Domain 2: Test Design							
U	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.			
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figure 1 and was adequate for the out- comes of interest.			
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups. The study was, however, reported to have a completely randomized design.			
Domain 3: Exposure Ch	aracterization						
	Metric 7:	Experimental System/Test Media Preparation	High	The test media was prepared by dissolving DBP into methanol to obtain 3 different con- centrations of stock solution. Stock solutions were added to cinnamon soil and stirred completely. They were then mixed with the appropriate amount of untreated soil to achieve the desired concentrations. The methanol was then allowed to evaporate with 5 day soil equilibration period.			
	Metric 8:	Consistency of Exposure Administration	High	All test containers were 18x16x22cm pots with 16 seeds each. All contained the same amount of treated soil or control soil under similar conditions.			
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured at any point.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be for 40 days with samples were taken at 14d, 24d and 40d. This was adequate to observe a response.			
	Metric 11:	Number of Exposure Groups/	Medium	There were only 3 exposure groups, which is lower than is typical, but the spacing was adequate to see a response			
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.			
Domain 4: Test Organisi	m						
6.00	Metric 13:	Test Organism Characteristics	High	The wheat seeds were from the Agro-Environmental Protection Institute, Ministry of Agriculture, China.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the seeds were acclimated.			
Continued on next page							

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HERO ID: 5495646 Table: 1 of 2

		conth	nucu nom p	Tevious page			
Study Citation:	Gao, M., Gu stages of wh	Gao, M., Guo, Z., Dong, Y., Song, Z. (2019). Effects of di-n-butyl phthalate on photosynthetic performance and oxidative damage in different growth stages of wheat in cinnamon soils. Environmental Pollution 250:357-365.					
Duration:	Overall Dura	tion: > 21 days; Exposure Duration: > 21	days				
Exposure Route,	Terrestrial; S	oil; Root uptake					
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Triticum aestivum L.; Emb	ryo				
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phthalate (DBP)						
HERO ID:	5495646						
Domain		Metric	Rating	Comments			
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 16 seeds per test chamber. There were 3 replicates for each treatment.			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	Medium	The plants were fertilized on a regular basis. They were kept in a greenhouse at 25C during the day and 15C at night in cinnamon soil. Little other information was provided. It would have been beneficial to have information on photoperiod and relative humidity.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant biomass.			
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups-at each sample period, plants were rinsed and dried with filter paper and separated into roots, stems, and leaves. Dry weights were then taken.			
Domain 6: Confounding	y / Variable Co	atrol					
Domain 0. Comoundang	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	The statistical methods were described in the "statistical analysis" section of the paper.			
	Metric 22:	Reporting of Data	High	The exposure and control outcomes were reported in Figure 1, and they were adequate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability is reported in Figure 1.			

Overall Quality Determination

High

Study Citation:	Gao, M., Guo, Z., Dong, Y., Song, Z. (2019). Effects of di-n-butyl phthalate on photosynthetic performance and oxidative damage in different growth							
Duration	stages of wh	stages of wheat in cinnamon soils. Environmental Pollution 250:357-365.						
Duration: Exposure Route.	Terrestrial: S	don: > 21 days; Exposure Duration: > 2.	1 days					
Media, Path:	Terrestriar, 5	ion, noor uptake						
Taxa, Species, Age:	Vegetation; V	Vegetation; Vascular Plants; Triticum aestivum L.; Embryo						
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis							
Chemical:	Dibutyl phth	Dibutyl phthalate (DBP)						
Domain	J49J040	Metric	Rating	Comments				
Domain 1: Test Substan	ce	Wiettie	Katilig	Comments				
	Metric 1:	Test Substance Identity	High	The DBP was identified by CASRN.				
	Metric 2:	Test Substance Source	Low	The source was reported to be J & K Scientific Ltd. in Beijing, China. However, it was not reported if the DBP was analytically verified.				
	Metric 3:	Test Substance Purity	Low	The purity of the DBP was not reported.				
Domain 2. T-+ D'								
Domain 2: Test Design	Metric 4.	Negative Controls	High	Study authors reported the use of a concurrent negative control				
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Figures 2-4 and in Table 1, and were				
			8	adequate for the outcomes of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups. The study was, however, reported to have a completely randomized design.				
Domain 3: Exposure Ch	aracterization							
Domain of Exposure on	Metric 7:	Experimental System/Test Media Preparation	High	The test media was prepared by dissolving DBP into methanol to obtain three differ- ent concentrations of stock solution. Stock solutions were added to cinnamon soil and stirred completely. They were then mixed with the appropriate amount of untreated soil to achieve the desired concentrations. The methanol was then allowed to evaporate with a 5-day soil equilibration period.				
	Metric 8:	Consistency of Exposure Administration	High	All test containers were 18x16x22cm pots with 16 seeds each. All contained the same amount of treated soil or control soil under similar conditions.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if the test concentrations were measured at any point.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be for 40 days with samples taken at 14d, 24d and 40d. This was adequate to observe a response.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only three exposure groups, which is lower than is typical, but the spacing was adequate to see a response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.				
Domain 4: Test Organis	m Metric 13:	Test Organism Characteristics	High	The wheat seeds were from the Agro-Environmental Protection Institute, Ministry of				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the seeds were acclimated.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were 16 seeds per test chamber. There were three replicates for each treatment.				
		Replicates per Group						
Continued on next page								

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HERO ID: 5495646 Table: 2 of 2

		conti	nued from p	revious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Gao, M., Guo, Z., Dong, Y., Song, Z. (2019). Effects of di-n-butyl phthalate on photosynthetic performance and oxidative damage in different growth stages of wheat in cinnamon soils. Environmental Pollution 250:357-365. Overall Duration: > 21 days; Exposure Duration: > 21 days Terrestrial; Soil; Root uptake Vegetation; Vascular Plants; <i>Triticum aestivum L.</i> ; Embryo Mechanistic-Oxidative stress (including redox biology)-Photosynthesis Dibutyl phthalate (DBP) 5495646					
Domain		Metric	Rating	Comments		
Domain 5: Outcome As	sessment Metric 16:	Adequacy of Test Conditions	Medium	The plants were fertilized on a regular basis. They were kept in a greenhouse at 25C during the day and 15C at night in cinnamon soil. Little other information was provided. It would have been beneficial to have information on photoperiod and relative humidity.		
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–changers in photosynthesis and oxidative responses.		
	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: Metric 20:	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 or other non-treatment-related factors across study groups. 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 of the paper.		
	Metric 22:	Reporting of Data	High	The exposure and control outcomes were reported in Figures 2-4 and in Table 1, and they were adequate for the outcomes of interest.		
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability is reported in the figures and table.		
Additional Comments:	This portion and oxidative	of the evaluation is on the effect of DBP on e stress were selected as the outcomes of ir	photosynthe nterest.	sis and oxidative stress mechanisms in wheat. Mechanistic outcomes of photosynthesis		

Overall Quality Determination

High

Study Citation:	Gao, M., Qi	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	uorescence of wheat seedlings. Chemosphere 151:76-83. verall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days							
Exposure Route,	Terrestrial; S	soil; Root uptake							
Media, Path:									
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported Mechanistic-Photosynthesis								
Health Outcome:	Mechanistic-Photosynthesis Dibutyl phthalate (DBP)								
HERO ID:	3350318	3350318							
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	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									
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 Ch	aracterization								
Domain 5. Exposure en	Metric 7:	Experimental System/Test Media	High	The experimental system and methods for preparation of test media were described in					
		Preparation	0	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/ 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.					
		Conditions	-						
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms (8 seedlings per concentration) were reported and suffi-					
		Replicates per Group		that the experiment was repeated five times.					
Domain 5: Outcome Ass	sessment								

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Environmental Hazard Evaluation

HERO ID: 3350318 Table: 1 of 2

		contin	ued 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 luorescence 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; Triticum sp; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Mechanistic	Photosynthesis						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	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	High	The outcome assessment methodologies for chlorophyll content and photosynthetic parameters were reported 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	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group				
		Design and Procedures		were not reported, but these are unlikely to have a substantial impact on results.				
	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. Data Present	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-				
	Matria 22		II:-h	ple comparisons were not provided.				
	Metric 22:	Exploration of Unexposted Outcome	High	Data for exposure-related findings were snown for each treatment and control group.				
	Metric 23:	Explanation of Unexpected Outcomes	High	I nere were no unexpected outcomes.				
Additional Comments:	The author's conclusions	discussion of the effects of DBP and D were made without incorporating any discu	EHP on gro	wth indices and mechanistic endpoints of wheat seedlings was not clear, and their istical significance. Results of multiple comparisons were not provided.				

Overall Quality Determination

High

PUBLIC RELEASE DRAFT May 2025 Environmental Hazard Evaluation

HERO ID: 3350318 Table: 2 of 2

Study Citation:	Gao, M., Qi	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 Overall Dura	luorescence of wheat seedlings. Chemosphere 151:76-83. Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
Exposure Route,	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:	Developmen	Development/Growth					
HERO ID:	3350318	3350318					
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	High	Chemical purity was reported as 96.8%.			
Domain 2: Test Design							
U	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						
r an r	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 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 Organist	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.			
	M . 1 17	Conditions					
	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 Acc	assmant			· · ·			
	5551110111						
		Conti	nued on next pa	ge			
Environmental Hazard Evaluation

HERO ID: 3350318 Table: 2 of 2

		contin	ued from previ	ous page					
Study Citation:	Gao, M., Q 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 Dur	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days							
Exposure Route,	Terrestrial; Soil; Root uptake								
Media, Path:									
Taxa, Species, Age:	Vegetation;	Vascular Plants; Triticum sp; Jinnong 7; Not	Applicable (e.g	., fungi or algae studies) or Not Reported					
Health Outcome:	Developme	nt/Growth							
Chemical:	Dibutyl phtl	halate (DBP)							
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 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 at days 7 and 14.					
Domain 6: Confounding	y / 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. 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	ation and Anal	lveie							
Domain 7. Data Present	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	s discussion of the effects of DBP and DEH g any discussion of statistical significance. R	IP on growth ind Results of multip	lices of wheat seedlings was not clear, and their conclusions were made without le comparisons were not provided.					
Overall Qualit	ty Deterr	nination	Medium						

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.						
Duration: Exposure Route, Media. Path:	Pedosphere 2 Overall Dura Terrestrial; S	Percenter 24(1):107-115. Dverall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Ferrestrial; 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; Vigna radiata; Not Applica	ble (e.g., fungi o	r algae studies) or Not Reported				
Health Outcome:	Reproductive	e/Teratogenic						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	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 99.1%				
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 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	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				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	response Exposure was via soil				
	incure 12.	Testing at of Below Solubility Ellint	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	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.				
Domain 5: Outcome Ass	sessment							
Domain 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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HERO ID: 2510954 Table: 1 of 3

		contin	ued from previo	bus page				
Study Citation:	Ma, T. T., C	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	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., c	hemical of intere	est in exposure water, but unable to determine exact uptake route)				
Media, Path:								
Taxa, Species, Age:	Vegetation;	Vascular Plants; Vigna radiata; Not Applicat	ble (e.g., fungi or	algae studies) or Not Reported				
Health Outcome:	Reproductiv	e/Teratogenic						
Chemical:	Dibutyl phth	alate (DBP)						
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						
	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:	Penorting of Data	Low	Date for exposure related findings were not shown for each treatment and control group				
	Metric 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:	This form is	to assess the germination outcome.						
Overall Quali	ty Deterr	nination	Medium					

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Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	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 Mechanistic-Biomarkers (exposure and effect) Dibutyl phthalate (DBP)					
HERO ID:	2510954					
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce		т			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Purity	L0W High	The test substance identity was not analytically verified by the performing laboratory.		
	mente 5.	rest Substance 1 utity	Ingii			
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 and 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 /:	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.		
		Administration		1 · · · · · · · · · · · · · · · · · · ·		
	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	8	response.		
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.		
Domain 4: Test Organis	m		TT' 1			
	Metric 13: Matria 14:	Lest Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.		
	Methe 14.	Conditions	LOW	The study did not report whether lest 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 Motrie 16	A doguogy of Tost Conditions	Մետե	Environmental conditions of the test system more an individual to minimum of a		
	Metric 10:	Aucquacy of Test Conditions	пıgn	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: 2510954 Table: 2 of 3

		contin	ued from p	previous page		
Study Citation:	Ma, T. T., Cł	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	24(1):107-115.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration	n: 0 - 4 days	s (0-96h)		
Exposure Route,	Terrestrial; S	soil; Not determined by study authors (i.e., c	chemical of	interest in exposure water, but unable to determine exact uptake route)		
Media, Path:						
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Vigna radiata; Not Applica	ble (e.g., fu	ngi or algae studies) or Not Reported		
Health Outcome:	Mechanistic	Biomarkers (exposure and effect)				
Chemical:	Dibutyl phth	alate (DBP)				
HERO ID:	2510954					
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	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 shown for each treatment and control group.		
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.		
Additional Comments:	s: Mechanistic endpoints are: malondialdehyde (MDA) Polyphenol oxidase (PPO)ascorbate peroxidase (APX)Superoxide dismutase (SOF)The glutathione (GSH)peroxidase (POD)Proline content					
Overall Quality Determination High						

Study Citation: Duration:	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)								
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: Health Outcome: Chemical: HERO ID:	Vegetation; V Developmen Dibutyl phth 2510954	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dibutyl phthalate (DBP) 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 99.1%					
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 groups was not reported					
	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	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:	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 adequate for a dose response					
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.					
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:	Conditions	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 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 Assessment	High	Outcomes were assessed consistently across study groups.					

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HERO ID: 2510954 Table: 3 of 3

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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.						
	Pedosphere	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	Soil; Not determined by study authors (i.e., cl	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 Applicab	le (e.g., fungi o	r algae studies) or Not Reported				
Health Outcome:	Developmen	nt/Growth						
Chemical:	Dibutyl phth	nalate (DBP)						
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	lysis						
	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:	Seedling root and shoot lengths were measured with a millimeter ruler, and the biomass (fresh weight, FW) in each dish was determined by weighing. Root length was defined as the length from root tip to root radicle.							

Overall Quality Determination

Medium

Study Citation:	Wang, S. G., Lin, X. G., Yin, R., Hou, Y. L. (2003). Effects of di-n-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants. Biologia Plantarum							
Duration	47(4):637-63	4/(4):637-639. Overall Duration: > 21 days: Exposure Duration: > 21 days						
Exposure Route	Terrestrial: S	Terrestrial: Soil: Root untake						
Media. Path:	Terresultar, 5	on, Root uptake						
Taxa, Species, Age:	Vegetation: V	Vascular Plants: Vigna sinensis: Juvenile						
Health Outcome:	Developmen	t/Growth						
Chemical:	Dibutyl phth	alate (DBP)						
HERO ID:	5495799							
Domain		Metric	Rating	Comments				
Domain 1: Test Substance	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 DBP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2: Test Design								
U	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control in which no DBP was added.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 2 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the seedlings were allocated into study groups.				
Domain 3: Exposure Ch	aracterization							
Bollium 5. Exposure en	Metric 7:	Experimental System/Test Media	Low	Little information was provided on the preparation of the test media.				
		Preparation		r				
	Metric 8:	Consistency of Exposure	Low	All exposure occurred for 60 days with samples taken at 20, 40, and 60 days. Test pot				
		Administration		dimensions were not described. More information needed to be reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if exposure concentrations were measured.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The exposure duration was reported to be 60 days. This was adequate to observe a re- sponse.				
	Metric 11:	Number of Exposure Groups/	Low	There were only two exposure levels, but the spacing was adequate to see a response.				
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	N/A	The exposure was via soil.				
Domain 4: Test Organisr	n							
2 sman 1. rost organisi	Metric 13:	Test Organism Characteristics	Low	Three-day old cowpea seedlings were used in the study, but the source of the cowpeas was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the plants were acclimated.				
	Metric 15:	Conditions Number of Organisms and	Medium	There were two cowpeas per test chamber, and there were three replicates per treatment.				
		Replicates per Group						

Domain 5: Outcome Assessment

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Study Citation:	Wang, S. G., 47(4):637-63	Wang, S. G., Lin, X. G., Yin, R., Hou, Y. L. (2003). Effects of di-n-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants. Biologia Plantarum 47(4):637-639.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Terrestrial; Soil; Root uptake						
Media, Path:							
Taxa, Species, Age:	Vegetation; V	Vascular Plants; Vigna sinensis; Juvenile					
Health Outcome:	Developmen	t/Growth					
Chemical:	Dibutyl phth	alate (DBP)					
HERO ID:	5495799						
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	Medium	The plants were kept on a 16L:8D photoperiod with temperatures at 22/18C for day/night. No information was provided on fertilizing or other environmental conditions.			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in root and shoot biomass.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the assessment of root and shoot biomass were limited.			
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. It was not re- ported if the plants 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 Present	ation and Anal	vsis					
	Metric 21:	Statistical Methods	Low	Statistics were performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for the control response and exposure response were reported in Table 2 and were appropriate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the			

Overall Quality Determination

Low

Study Citation:	Wang, S. G., 47(4):637-63	Wang, S. G., Lin, X. G., Yin, R., Hou, Y. L. (2003). Effects of di-n-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants. Biologia Plantarum 47(4):637-639.						
Duration: Exposure Route, Media. Path:	Overall Dura Terrestrial; S	verall Duration: > 21 days; Exposure Duration: > 21 days errestrial; Soil; Root uptake						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; V ADME (biot Dibutyl phth 5495799	Vascular Plants; <i>Vigna sinensis</i> ; Juvenile ransformation) alate (DBP)						
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 DBP was not reported.				
	Metric 3:	Test Substance Purity	Low	The purity/grade of the DBP was not reported.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control in which no DBP was added.				
	Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3 and was adequate for the outcome of interest.				
	Metric 6:	Randomized Allocation	Low	It was not reported how the seedlings were allocated into study groups.				
Domain 3: Exposure Cha	aracterization							
	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test media.				
	Metric 8:	Consistency of Exposure Administration	Low	All exposure occurred for 60 days with samples taken at 20, 40, and 60 days. Test pot dimensions were not described. More information needed to be reported.				
	Metric 9:	Measurement of Test Substance	Low	It was not reported if exposure concentrations were measured.				
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 60 days. This was adequate to observe a re- sponse.				
	Metric 11:	Number of Exposure Groups/	Low	There were only 2 exposure levels, but the spacing was adequate to see a response.				
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.				
Domain 4. Test Organise	n							
	Metric 13:	Test Organism Characteristics	Low	3 day old cowpea seedlings were used in the study, but the source of the cowpeas was not reported.				
	Metric 14:	Acclimatization and Pretreatment	Low	It was not reported if the plants were acclimated.				
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	There were two cowpeas per test chamber, and there were 3 replicates per treatment.				
Domain 5: Outcome Ass	sessment Metric 16:	Adequacy of Test Conditions	Medium	The plants were kept on a 16L:8D photoperiod with temperatures at 22/18C for day/night. No information was provided on fertilizing or other environmental conditions.				
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Study Citation:	Wang, S. G. 47(4):637-6	Wang, S. G., Lin, X. G., Yin, R., Hou, Y. L. (2003). Effects of di-n-butyl phthalate on mycorrhizal and non-mycorrhizal cowpea plants. Biologia Plantarum 47(4):637-639.					
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days						
Exposure Route,	Terrestrial; S	Soil; Root uptake					
Media, Path:							
Taxa, Species, Age:	Vegetation;	Vascular Plants; Vigna sinensis; Juvenile					
Health Outcome:	ADME (bio	transformation)					
Chemical:	Dibutyl phth	nalate (DBP)					
HERO ID:	5495799						
Domain		Metric	Rating	Comments			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DBP accumulation in plant tissues.			
	Metric 18:	Consistency of Outcome Assessment	High	The shoots and roots were oven dried and ground up for DBP analysis. Gas chromatog- raphy was used to determine DBP levels in the tissues.			
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. It was not re- ported if the plants 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 Presen	tation and Anal	lysis					
	Metric 21:	Statistical Methods	Low	Statistics were performed but not described adequately.			
	Metric 22:	Reporting of Data	High	Data for the control response and exposure response were reported in Table 3 and were appropriate for the outcomes of interest.			
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report and unexpected outcomes. Variability was reported in the table.			
Additional Comments:	This portion	of the evaluation was on DBP accumulation	in the roots and	shoots of cowpeas. ADME was selected as the outcome of interest.			
Overall Quali	ty Deterr	nination	Medium				