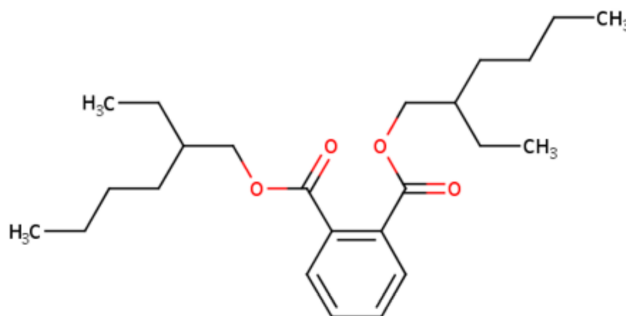


**Data Quality Evaluation Information for  
Environmental Hazard for  
Diethylhexyl Phthalate (DEHP)  
(1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester)**

**Systematic Review Support Document for the Risk Evaluation**

**CASRN: 117-81-7**



*December 2025*

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

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

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<b>Diethylhexyl Phthalate</b>		
<b>Habitat: Aquatic (freshwater)</b>		
<b>Taxa: Vertebrates</b>		
<i>Carassius auratus</i>		
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<i>Clarius gariepinus</i>		
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<i>Cyprinus carpio</i>		
5554274	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. <i>Journal of Environmental Sciences</i> 46:47-54.	47
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<i>Danio rerio</i>		

<b>2298079</b>	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. <i>International Journal of Environmental Research and Public Health</i> 11(3):3156-3168.	<b>54</b>
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<b>3071151</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). <i>Environmental Pollution</i> 203(Elsevier):130-136.	<b>112</b>
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<i>Lampetra planeri</i>		
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>124</b>
<i>Lepomis macrochirus</i>		
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<b>11328252</b>	Kirsch, P., Munk, R. (1989). Report on the study of the acute toxicity.	<b>138</b>
	<i>Oncorhynchus mykiss</i>	
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	<i>Oncorhynchus mykiss (Salmo gairdneri)</i>	
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	<i>Pelteobagrus fulvidraco</i>	
<b>4742097</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 202:79-84.	<b>209</b>
	<i>Phoxinus phoxinus</i>	
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>221</b>
	<i>Pimephales promelas</i>	
<b>1321996</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.	<b>223</b>
<b>1316188</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.	<b>227</b>
<b>1316189</b>	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows ( <i>Pimephales promelas</i> ) under flow-through conditions.	<b>230</b>
<b>5774391</b>	Defoe, D. L., Holcombe, G. W., Hammermeister, D. E., Biesinger, K. E. (1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. <i>Environmental Toxicology and Chemistry</i> 9(5):623-636.	<b>234</b>
<b>3071071</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.	<b>236</b>
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	<i>Pungitius pungitius</i>	
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	<i>Salmo mykiss</i>	
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<b>5678430</b>	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon ( <i>Salmo salar</i> ) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.	<b>350</b>
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<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. Environmental Pollution 27(4):263-274.	<b>394</b>
<b>813673</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.	<b>396</b>
<b>1332972</b>	Streufert, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).	<b>400</b>
	<i>Chironomus riparius</i>	



<b>3859131</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). <i>PLoS ONE</i> 12(2):e0171719.	<b>404</b>
<b>2519014</b>	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during <i>Chironomus riparius</i> development. <i>Science of the Total Environment</i> 470-471:1003-1011.	<b>410</b>
<b>681990</b>	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in <i>Chironomus riparius</i> exposed to di-2-ethylhexyl phthalate (DEHP). <i>Journal of Environmental Biology</i> 25(3):259-261.	<b>413</b>
<b>681634</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . <i>Bulletin of Environmental Contamination and Toxicology</i> 74(6):1179-1185.	<b>417</b>
<i>Chironomus tentans</i>		
<b>492760</b>	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in <i>Chironomus tentans</i> (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. <i>Chemosphere</i> 65(6):1074-1081.	<b>423</b>
<b>1335360</b>	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to <i>Chironomus tentans</i> .	<b>427</b>
<b>674438</b>	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. <i>Environment International</i> 33(6):817-822.	<b>431</b>
<b>679311</b>	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. <i>Environmental Toxicology and Chemistry</i> 20(8):1805-1815.	<b>433</b>
<b>679312</b>	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. <i>Environmental Toxicology and Chemistry</i> 20(8):1798-1804.	<b>437</b>
<i>Daphnia magna</i>		
<b>1321996</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.	<b>439</b>
<b>1316223</b>	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to <i>Daphnia magna</i> (final report).	<b>441</b>
<b>679904</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . <i>Chemosphere</i> 36(6):1367-1379.	<b>444</b>
<b>1334281</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. <i>Chemosphere</i> 11(4):417-426.	<b>446</b>
<b>5750702</b>	Huang, B., Li, D., Yang, Y. (2016). Joint toxicity of two phthalates with waterborne copper to <i>Daphnia magna</i> and <i>Photobacterium phosphoreum</i> . <i>Bulletin of Environmental Contamination and Toxicology</i> 97(3):380-386.	<b>448</b>
<b>789536</b>	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. <i>Environmental Toxicology and Chemistry</i> 22(12):3037-3043.	<b>450</b>
<b>3070913</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . <i>Science of the Total Environment</i> 545-546(Elsevier):127-136.	<b>452</b>

<b>1335345</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> .	<b>462</b>
<b>1335353</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> in the presence of fulvic acid.	<b>464</b>
<b>11328251</b>	Muller (1983). Determination of the acute toxicity of di-2-ethylhexyl-phthlat (dehp) to the waterflea <i>daphnia magna</i> straus.	<b>466</b>
<b>674438</b>	Park, S. Y., Choi, J. (2007). Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. <i>Environment International</i> 33(6):817-822.	<b>468</b>
<b>2966135</b>	Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator <i>Daphnia magna</i> indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. <i>Environmental Science &amp; Technology</i> 49(12):7400-7410.	<b>470</b>
<b>5043468</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . <i>Science of the Total Environment</i> 654:969-977.	<b>475</b>
<b>5498837</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . <i>Archives of Environmental Contamination and Toxicology</i> 75(1):145-156.	<b>485</b>
<b>1334646</b>	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. <i>Environmental Research</i> 6(1):84-90.	<b>497</b>
<b>679904</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . <i>Chemosphere</i> 36(6):1367-1379.	<b>499</b>
<b>1334281</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. <i>Chemosphere</i> 11(4):417-426.	<b>505</b>
<b>1334646</b>	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. <i>Environmental Research</i> 6(1):84-90.	<b>511</b>
<b>1334951</b>	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of <i>daphnia-magna</i> . <i>Environmental Toxicology and Chemistry</i> 6(3):201-208.	<b>513</b>
<b>680120</b>	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to <i>Daphnia magna</i> and rainbow trout ( <i>Oncorhynchus mykiss</i> ). <i>Environmental Toxicology and Chemistry</i> 14(11):1967-1976.	<b>521</b>
<b>5043468</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . <i>Science of the Total Environment</i> 654:969-977.	<b>525</b>
<b>1316195</b>	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to <i>Daphnia magna</i> with cover letter dated 032585. :95.	<b>527</b>
<i>Dendrocoelum lacteum</i>		
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>531</b>
<i>Gammarus pseudolimnaeus</i>		

<b>1334646</b>	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. <i>Environmental Research</i> 6(1):84-90.	<b>533</b>
<b>1334646</b>	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. <i>Environmental Research</i> 6(1):84-90.	<b>535</b>
<i>Gammarus pulex</i>		
<b>1335277</b>	Oil, Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp <i>Gammarus pulex</i> (L) to toxicants with cover letter.	<b>537</b>
<b>732821</b>	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod <i>Gammarus pulex</i> . <i>Bulletin of Environmental Contamination and Toxicology</i> 46(1):159-166.	<b>539</b>
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>545</b>
<i>Helobdella sp.</i>		
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>547</b>
<i>Hexagenia bilineata</i>		
<b>1334646</b>	Jr, Mayer, F., Sanders, H. O., Walsh, D. F. (1973). Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. <i>Environmental Research</i> 6(1):84-90.	<b>549</b>
<i>Hyaella azteca</i>		
<b>679311</b>	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. <i>Environmental Toxicology and Chemistry</i> 20(8):1805-1815.	<b>551</b>
<b>679312</b>	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. <i>Environmental Toxicology and Chemistry</i> 20(8):1798-1804.	<b>555</b>
<i>Limnephilus sp</i>		
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>557</b>
<i>Lumbriculus variegatus</i>		
<b>679312</b>	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. <i>Environmental Toxicology and Chemistry</i> 20(8):1798-1804.	<b>559</b>
<i>Macrobrachium rosenbergii</i>		
<b>789598</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . <i>Aquatic Toxicology</i> 64(1):25-37.	<b>561</b>
<i>Paratanytarsus parthenogenetica</i>		

<b>1321996</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.	<b>567</b>
<b>1335357</b>	Monsanto, (1983). Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge <i>Paratanytarsus parthenogenetica</i> .	<b>569</b>
	<i>Paratanytarsus parthenogenetica</i>	
<b>1316219</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to <i>Paratanytarsus parthenogenetica</i> (final report) report no BW-83-6-1424.	<b>571</b>
	<i>Planorbis corneus</i>	
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>573</b>
	<i>Sialis sp.</i>	
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>575</b>
	<i>Tubijex sp.</i>	
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>577</b>
<b>Taxa: Plants (Non-vascular)</b>		
	<i>Chlorella vulgaris</i>	
<b>679344</b>	Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in <i>Chlorella vulgaris</i> . <i>Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances &amp; Environmental Engineering</i> 42(2):179-183.	<b>579</b>
<b>5692135</b>	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga <i>Chlorella vulgaris</i> . <i>IOP Conference Series: Earth and Environmental Science</i> 227(5):052054.	<b>581</b>
	<i>Pseudokirchneriella subcapitata</i>	
<b>789536</b>	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. <i>Environmental Toxicology and Chemistry</i> 22(12):3037-3043.	<b>585</b>
	<i>Selenastrum capricornutum</i>	
<b>1321996</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.	<b>587</b>
<b>1316196</b>	Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga <i>Selenastrum capricornutum</i> .	<b>589</b>

**Taxa: Plants (Vascular)***Chara chara*

<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>591</b>
	<i>Lemna minor</i>	
<b>1340050</b>	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.	<b>593</b>
	<i>Mentha aquatica</i>	
<b>59542</b>	Sodergren, A. (1982). Significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in an aquatic laboratory model ecosystem. <i>Environmental Pollution</i> 27(4):263-274.	<b>595</b>
	<i>Spirodela polyrhiza</i>	
<b>1340050</b>	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.	<b>597</b>
	<i>Triticum sp.</i>	
<b>3515118</b>	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. <i>Chemosphere</i> 172(Elsevier):418-428.	<b>599</b>
<b>3515118</b>	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. <i>Chemosphere</i> 172(Elsevier):418-428.	<b>601</b>

## Habitat: Aquatic (marine)

### Taxa: Vertebrates

	<i>Cyprinodon variegatus</i>	
<b>18110</b>	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows ( <i>Cyprinodon variegatus</i> ). <i>Bulletin of Environmental Contamination and Toxicology</i> 27(5):596-604.	<b>607</b>
	<i>Oryzias melastigma</i>	
<b>2519010</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.	<b>609</b>
	<i>sheepshead minnow (Cyprinodon variegatus)</i>	
<b>1316224</b>	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).	<b>615</b>

### Taxa: Invertebrates

	<i>Haliotis diversicolor supertexta</i>	
<b>697762</b>	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 <i>Haliotis diversicolor supertexta</i> . <i>Ecotoxicology</i> 18(3):293-303.	<b>617</b>

<b>1322103</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.	<b>619</b>
<b>1249532</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . Environmental Pollution 159(5):1114-1122.	<b>621</b>
<b>1322103</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.	<b>627</b>
<i>Macrophthalmus japonicus</i>		
<b>5567571</b>	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, <i>Macrophthalmus japonicus</i> . Fish and Shellfish Immunology 87:322-332.	<b>629</b>
<i>Mysidopsis bahia</i>		
<b>1321996</b>	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.	<b>633</b>
<b>1316220</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp ( <i>Mysidopsis bahia</i> ).	<b>635</b>
<i>Mytilus edulis</i>		
<b>1334379</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 2. The bioconcentration and depuration of di-2-ethylhexyl phthalate and diisodecyl phthalate in mussels, ( <i>Mytilus edulis</i> ). Chemosphere 11(4):427-435.	<b>637</b>
<i>PALAEMONETES PUGIO</i>		
<b>1333217</b>	RB, Laughlin, J. R., Neff, J. M., Hrun, Y. C., Goodwin, T. C., Giam, C. S. (1978). The effects of three phthalate esters on the larval development of the grass shrimp <i>Palaemonetes pugio</i> (Holthuis). Water, Air, and Soil Pollution 9(3):323-336.	<b>639</b>
<i>Parvocalanus crassirostris</i>		
<b>3859142</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . Ecotoxicology and Environmental Safety 141:298-305.	<b>645</b>
<b>3859142</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . Ecotoxicology and Environmental Safety 141:298-305.	<b>649</b>
<b>3859142</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . Ecotoxicology and Environmental Safety 141:298-305.	<b>655</b>
<i>Penaeus vannamei</i>		
<b>679685</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.	<b>657</b>

<b>679685</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.	<b>659</b>
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### Taxa: Plants (Non-vascular)

#### *Karenia brevis*

<b>3230225</b>	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on <i>Karenia brevis</i> . Chemosphere 155:498-508.	<b>665</b>
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### Habitat: Aquatic (brackish)

### Taxa: Vertebrates

#### *Cyprinodon variegatus*

<b>789995</b>	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.	<b>668</b>
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#### *sheepshead minnow (Cyprinodon variegatus)*

<b>1316224</b>	Bionomics., Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).	<b>670</b>
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### Taxa: Invertebrates

#### *Artemia salina*

<b>1315792</b>	Sugawara, N. (1974). Toxic effect of a normal series of phthalate esters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-89.	<b>672</b>
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#### *Crassostrea virginica*

<b>789995</b>	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.	<b>674</b>
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#### *Eurytemora affinis*

<b>679508</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.	<b>676</b>
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<b>679508</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). Ecotoxicology and Environmental Safety 60(3):288-294.	<b>680</b>
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#### *Mysidopsis bahia*

<b>1316220</b>	Bionomics., EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp ( <i>Mysidopsis bahia</i> ).	<b>682</b>
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#### *Nitocra spinipes*

<b>51937</b>	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 ( <i>Alburnus alburnus</i> ) and the harpacticoid <i>Nitocra spinipes</i> . <i>Chemosphere</i> 8(11-12):843-851.	<b>684</b>
	<i>Penaecus aztecus</i>	
<b>789995</b>	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. <i>Ecotoxicology and Environmental Safety</i> 5(2):202-210.	<b>686</b>
<b>Habitat: Terrestrial</b>		
<b>Taxa: Vertebrates</b>		
	<i>Bos taurus, Holstein Fresian</i>	
<b>3071101</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. <i>PLoS ONE</i> 10(7):e0130896.	<b>688</b>
	<i>Common marmosets (Callithrix jacchus)</i>	
<b>630680</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. <i>Toxicological Sciences</i> 42(1):49-56.	<b>694</b>
	<i>Coturnix japonica</i>	
<b>6816734</b>	Wang, H., Guan, T. Q., Sun, J. X., Talukder, M., Huang, Y. Q., Li, Y. H., Li, J. L. (2020). Di-(2-ethylhexyl) phthalate induced nephrotoxicity in quail ( <i>Coturnix japonica</i> ) by triggering nuclear xenobiotic receptors and modulating the cytochrome P450 system. <i>Environmental Pollution</i> 261:114162.	<b>706</b>
	<i>Gallus domesticus</i>	
<b>683058</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. <i>Lipids</i> 15(3):151-156.	<b>710</b>
	<i>Gallus gallus domesticus</i>	
<b>1249807</b>	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. <i>Neurotoxicology and Teratology</i> 34(1):56-62.	<b>718</b>
	<i>Ovis aries</i>	
<b>2519005</b>	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. <i>Environmental Toxicology and Pharmacology</i> 36(3):1141-1149.	<b>726</b>
	<i>Putorius putorius</i>	
<b>746754</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. <i>Toxicology</i> 6(3):341-356.	<b>732</b>
	<i>Streptopelia risoria</i>	



<b>681729</b>	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.	<b>740</b>
	<i>Sus domesticus</i>	
<b>683666</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.	<b>742</b>
	<i>Sus scrofa</i>	
<b>683808</b>	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.	<b>750</b>
<b>Taxa: Invertebrates</b>		
	<i>Caenorhabditis elegans</i>	
<b>5593882</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.	<b>756</b>
<b>5555457</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in Caenorhabditis elegans. Science of the Total Environment 634:260-266.	<b>764</b>
<b>4728405</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	<b>773</b>
<b>698288</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode Caenorhabditis elegans. Toxicology 237(1-3):126-133.	<b>781</b>
<b>5043459</b>	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. PLoS Genetics 15(2):e1007975.	<b>789</b>
<b>2215375</b>	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.	<b>791</b>
<b>4829298</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in Caenorhabditis elegans. Ecotoxicology and Environmental Safety 163:298-306.	<b>809</b>
<b>5593882</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in Caenorhabditis elegans. Environmental Pollution 251:871-878.	<b>815</b>
<b>4728405</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	<b>817</b>
<b>4728405</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of Caenorhabditis elegans. Chemosphere 190:375-382.	<b>819</b>
	<i>Drosophila melanogaster</i>	

<b>5495570</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . <i>Basic &amp; Clinical Pharmacology &amp; Toxicology Online Pharmacology Online</i> 119(3):309-316.	<b>821</b>
<b>5495717</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . <i>Chemosphere</i> 221:493-499.	<b>827</b>
<b>5494836</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.	<b>829</b>
<b>200657</b>	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a <i>drosophila</i> assay predominantly monitoring interchromosomal mitotic recombination. <i>Mutagenesis</i> 8(1):57-81.	<b>838</b>
<b>5495717</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . <i>Chemosphere</i> 221:493-499.	<b>841</b>
<b>5494836</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.	<b>845</b>
<b>5494836</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.	<b>851</b>
<i>Eisenia fetida</i>		
<b>3625226</b>	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 <i>Eisenia fetida</i> . <i>Journal of Environmental Quality</i> 14(3):383-388.	<b>857</b>
<i>Folsomia fimetaria</i>		
<b>789786</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . <i>Environmental Toxicology and Chemistry</i> 20(5):1085-1091.	<b>859</b>
<b>789786</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . <i>Environmental Toxicology and Chemistry</i> 20(5):1085-1091.	<b>861</b>
<b>789786</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . <i>Environmental Toxicology and Chemistry</i> 20(5):1085-1091.	<b>865</b>
<i>Lasius niger</i>		
<b>2345940</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . <i>Environmental Research</i> 131:104-110.	<b>869</b>
<b>2347468</b>	Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. <i>Environmental Science and Pollution Research</i> 21(23):13446-13451.	<b>875</b>
<b>2345940</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . <i>Environmental Research</i> 131:104-110.	<b>877</b>
<i>Spodoptera littoralis</i>		

<b>5494137</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siauxsat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . <i>Chemosphere</i> 215:725-738.	<b>879</b>
<b>Taxa: Plants (Vascular)</b>		
<i>Allium cepa</i>		
<b>1249401</b>	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the <i>Allium cepa</i> test. <i>Mutation Research</i> 743(1-2):20-24.	<b>893</b>
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>897</b>
<i>Avena sativa</i>		
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>899</b>
<i>Benincasa hispida</i>		
<b>2215486</b>	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant <i>Benincasa hispida</i> and its use for lowering DEHP content of intercropped vegetables. <i>Journal of Agricultural and Food Chemistry</i> 61(22):5220-5225.	<b>905</b>
<i>Cucumis sativus</i>		
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>909</b>
<b>1987637</b>	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. <i>Environmental Science and Pollution Research</i> 21(2):1020-1028.	<b>915</b>
<i>Lolium perenne</i>		
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>919</b>
<i>Medicago sativa</i>		
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>925</b>
<i>Nicotiana tabacum</i>		
<b>5627041</b>	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. <i>Pedosphere</i> 27(6):1073-1082.	<b>931</b>
<i>Nicotinana tobacum</i>		
<b>792357</b>	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. <i>Allelopathy Journal</i> 27(1):87-96.	<b>935</b>
<i>Raphanus sativus</i>		

<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>943</b>
	<i>Triticum aestivum</i>	
<b>2915866</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.	<b>949</b>
<b>5493185</b>	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. <i>Chemosphere</i> 209:258-267.	<b>955</b>
	<i>Triticum sp</i>	
<b>3350318</b>	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. <i>Chemosphere</i> 151:76-83.	<b>959</b>
	<i>Vigna radiata</i>	
<b>2510954</b>	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. <i>Pedosphere</i> 24(1):107-115.	<b>963</b>

<b>Study Citation:</b>	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Liver toxicology-Reproductive/Teratogenic-Nutritional and Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249842			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was reported to be Sigma Aldrich, but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the DEHP was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control in which the vehicle solvent, DMSO, was used at the same concentration as the exposure.	
	Metric 5: Negative Control Response	High	Table 1 provides P-values for every treatment-to-treatment comparison for male livers and gonads; this includes the comparison between DEHP and the control. Figure 1 presents control response on the metabolic profiles of the liver and the gonads. Figure S4A provided heatmaps of control based z-scores of VIP>1 metabolites for both the liver and the gonad.	
	Metric 6: Randomized Allocation	Low	It was not reported how the goldfish were allocated to each tank. However, it was reported that each treatment group was randomly assigned to each tank to avoid bias.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Study authors reported the test substances were added to the water every 24h after the tanks were drained to approximately 10% volume and then refilled with fresh water. Therefore the fish were exposed to declining concentrations of contaminants throughout each day of the study. Study authors also reported actual concentrations of the chemicals were not measured over time. A flow rate of 300mL/min of carbon-filtered City of Calgary water passed through each tank.	
	Metric 8: Consistency of Exposure Administration	Low	The size of the test chambers was not reported, though holding/storage tanks were reported to be 49L. It was unclear if these were the same tanks used in the study. All fish were held at 17C. However, study authors admitted that the fish were exposed to declining chemical concentrations over a 24h period until the solution was renewed. These test concentrations were not measured over time creating concern regarding the consistency of the study.	
	Metric 9: Measurement of Test Substance Concentration	Low	Study authors reported that the test chemicals were not measured over time in the study and that the test chemicals would decline over a 24h period.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 10d. This appeared adequate to see a response.	
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<b>Study Citation:</b>	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. Journal of Proteome Research 11(2):1133-1143.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Liver toxicology-Reproductive/Teratogenic-Nutritional and Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249842			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level, as the goal of the study was to compare the response of different test chemicals to each other and a control as well as to a mixture of the test substances.	
	Metric 12: Testing at or Below Solubility Limit	High	The DEHP exposure concentration was below the water solubility limit, and DMSO was used a vehicle solvent. An appropriate solvent control was used.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The goldfish were reported to be from Aquatic Imports in Calgary, Alberta, Canada. The age of the goldfish was not reported, though they were reported to be ~10cm long and 30g in weight.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The fish were reported to be acclimated for 72h prior to the start of the study.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Study authors reported there were 20 fish per exposure aquaria. Only the male goldfish were used for this study, and there were 6-8 males in each tank. 6 males were used for assessment for each chemical. The treatment aquaria were not replicated due to practical limitations.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	The goldfish were fed daily for the duration of the holding and study period. They were held at 17C in 49L tanks for the acclimation period with 16-18 fish per tank. There were 20 fish in each exposure aquaria, but the size of the exposure aquaria was not reported. Carbon-filtered City of Calgary water was used in the study, but water quality parameters were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. H NMR was used to assess various changes in metabolite levels due to DEHP exposure in the male goldfish liver and gonads relative to the controls.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Detailed description of the metabolite extraction and H MNR spectroscopy were provided. Analysis methods of the data were also described in detail.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	More information on the actual study environmental conditions is needed, though the goldfish were reported to be acclimated to test conditions.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
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<b>Study Citation:</b>	Jordan, J., Zare, A., Jackson, L. J., Habibi, H. R., Weljie, A. M. (2012). Environmental contaminant mixtures at ambient concentrations invoke a metabolic stress response in goldfish not predicted from exposure to individual compounds alone. <i>Journal of Proteome Research</i> 11(2):1133-1143.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Liver toxicology-Reproductive/Teratogenic-Nutritional and Metabolic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1249842		
Domain	Metric	Rating	Comments
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Detailed description of the data analysis was provided in 3 sections in the paper, "H NMR Data Analysis," "Statistical Analysis," and "Pathway Analysis."
	Metric 22: Reporting of Data	High	O-PLS-DA analysis score plots were provided for treatment and control outcomes in Fig 1 as well as in Fig S3. Z-score plots on the effect of DEHP on each metabolite in comparison to the control was provided in Fig. S2. DEHP heatmaps in comparison to the control were provided in Fig. S4. Fig S5 B provides the SUS plots, and metabolite pathways affected by the treatments are provided in Fig S6. The effect on Canonical pathways is provided in Fig S7. Metabolic disturbances based on metabolites and their superpathways is are provided in Fig 3. Canonical pathways and biological pathways that were affected by the exposure are provided in Fig. 4.
	Metric 23: Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes.
<b>Additional Comments:</b>	This evaluation was on the effect of DEHP on the metabolic profiles of male goldfish livers and testis. H NMR was used to assess metabolite identification and quantification. Mechanistic outcomes for metabolism, liver, reproduction, and endocrine were selected as the outcomes of interest.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. <i>Aquatic Toxicology</i> 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was only identified by name. CASRN, structure, or other chemical descriptors were not provided.	
	Metric 2: Test Substance Source	Low	The test source was not reported, and the substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity of DEHP was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the 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 the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment methodology reported the intended outcome of interest.	
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	High	There were no differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical methods were adequately described.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None			
Overall Quality Determination		High		

<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. <i>Aquatic Toxicology</i> 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the 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 the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
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<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. <i>Aquatic Toxicology</i> 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment methodology reported the intended outcome of interest.	
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	High	There were no differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical methods were adequately described.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. <i>Aquatic Toxicology</i> 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was only identified by name. CASRN, structure, or other chemical descriptors were not provided.	
	Metric 2: Test Substance Source	Low	The test source was not reported, and the substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity of DEHP was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 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 the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There were uncertainties about the source of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
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<b>Study Citation:</b>	Golshan, M., Hatef, A., Socha, M., Milla, S., Butts, I. A., Carnevali, O., Rodina, M., Sokołowska-Mikołajczyk, M., Fontaine, P., Linhart, O., Alavi, S. M. (2015). Di-(2-ethylhexyl)-phthalate disrupts pituitary and testicular hormonal functions to reduce sperm quality in mature goldfish. Aquatic Toxicology 163:16-26.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966358			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment methodology reported the intended outcome of interest.
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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The statistical methods were adequately described.
	Metric 22:	Reporting of Data	Medium	Brief results were described in the text. Data for the development/growth parameters are included in supplemental material that was not part of this pdf.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This evaluation is for the Development/Growth parameters (total length, body mass, GSI, and HSI) assessed.			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Juvenile			
<b>Health Outcome:</b>	Cardiovascular			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333101			
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	The purity and/or grade of test substance were not reported.
Domain 2: Test Design				
	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 Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 8:	Consistency of Exposure Administration	Uninformative	It was reported that a sonicated emulsion was used for exposure, and exposure administration cannot be administered consistently across test organisms/study groups using emulsions.
	Metric 9:	Measurement of Test Substance Concentration	Uninformative	Exposure concentrations were not measured, and nominal values are highly uncertain due to the nature of the test substance.
	Metric 10:	Exposure Duration and Frequency	Uninformative	The duration of exposure and/or exposure frequency were not reported.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and the spacing of exposure levels. It was reported "no concentration of DEHP tried was effective," and the concentrations specified on Fig 6 are not legible.
	Metric 12:	Testing at or Below Solubility Limit	Low	An emulsion was used so there is a high probability it exceeds solubility.
Domain 4: Test Organism				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and/or replicates were not reported. The figure on heart rate activity (Fig 6) showed only the response of an individual fish. The cited reference for methods (Francis et al., 1975) was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Carassius auratus</i> ; Juvenile		
<b>Health Outcome:</b>	Cardiovascular		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1333101		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. The cited reference for methods (Francis et al., 1975) was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Domain 6: Confounding / 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 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	Uninformative	Statistical analysis was not conducted.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown, but the results were described in the text.
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) or sufficient information to determine if excessive variability or unexpected outcomes occurred.
Additional Comments:	Overall, a poorly designed and poorly written study. Negative control group was not reported. Details of the experimental system, test media preparation, exposure concentrations, duration of exposure were not provided. It was reported that a sonicated emulsion was used for exposure. Mean heart rate data was not provided for DEHP, and the figure that shows the heart rate activity of one individual fish was of poor resolution. The cited reference for methods was not available at the time of review.		
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
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.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.	
	Metric 8: Consistency of Exposure Administration	Medium	Reporting omissions were unlikely to have a substantial impact on results.	
	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 the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were the same for the control and the exposed organisms, although few details were provided.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.	
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<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharp-tooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
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 / 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
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.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Medium	Reporting omissions were unlikely to have a substantial impact on results.
	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 the spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were the same for control and exposed organisms, although few details were provided.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
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<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	4829324		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
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.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	Medium	Reporting omissions were unlikely to have a substantial impact on results.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were the same for the control and the exposed organisms, although few details were provided.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
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<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	4829324		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
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.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.	
	Metric 8: Consistency of Exposure Administration	Medium	Reporting omissions were unlikely to have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured other than in tissue.	
	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 the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were the same for the control and the exposed organisms, although few details were provided.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of the test system were acceptable for the maintenance of organism health, although few details were provided.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
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<b>Study Citation:</b>	Adeogun, A. O., Ibor, O. R., Imiuwa, M. E., Omogbemi, E. D., Chukwuka, A. V., Omiwole, R. A., Arukwe, A. (2018). Endocrine disruptor responses in African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to di-(2-ethylhexyl)-phthalate. <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 213:7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarias gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829324			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
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	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: Reporting of Data	Low	Data for exposure-related findings were not reported 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**

<b>Study Citation:</b>	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endocrinology 254:22-37.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494023			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DEHP was identified by CASRN.	
	Metric 2: Test Substance Source	High	The source of the DEHP was reported to be from Tokyo Chemical Industry in Japan, and it was verified by GC-MSD.	
	Metric 3: Test Substance Purity	High	The DEHP purity was reported to be 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used. Ethanol was the reported vehicle solvent in the treatments.	
	Metric 5: Negative Control Response	High	The negative control response was reported and adequate for the outcomes of interest. The negative control responses can be found in the text in section 3.1.	
	Metric 6: Randomized Allocation	Medium	It was reported the fish were randomly allocated into 10 120L tanks with 60L of dechlorinated tap water with 50 fish per exposure group.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The system was reported to be a static renewal system with the test medium renewed every third day for the duration of the study. The stock solution was prepared by dissolving 10mL of DEHP in 90mL of ethanol to get 109.5g/L DEHP. Test concentrations were prepared through serial dilutions with dechlorinated tap water.	
	Metric 8: Consistency of Exposure Administration	High	All exposures were in 120L tanks with 50 fish per tank. Each tank had 60L of test solution that was diluted with dechlorinated tap water. All tests were conducted at 24C.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the exposure concentrations were measured at any point in the study.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be 14 days. This was adequate to observe a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 4 exposure groups. The spacing was adequate to see a response in DEHP accumulation in the liver according to dose.	
	Metric 12: Testing at or Below Solubility Limit	High	The highest concentration tested was above the water solubility limit of DEHP. However, ethanol was used as a vehicle solvent, and the solvent control results were adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The <i>C. gariepinus</i> were reported to be from the hatchery unit of the Aquaculture Department at the University of Ibadan. They were reported to be fingerlings that were 4 weeks old.	
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<b>Study Citation:</b>	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to waterborne di-(2-ethylhexyl) phthalate. General and Comparative Endocrinology 254:22-37.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494023			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The fish were held at 24C with a pH of 7 and a DO of 3mg/L. A natural photoperiod was reported to be used, but was not specified. The fish were fed twice daily throughout the experiment. Other properties of the test water were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DEHP accumulation in the liver of treated fish.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. EPA DIN 19742 was cited for the methodology for determining tissue levels. This method used was gas chromatography/mass selective detector for assessment.
Domain 6: Confounding / 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	High	All data were presented as +- mean standard deviation and were analyzed using Prism GraphPad 5. Significant differences between the solvent control and exposure groups were performed using one-way ANOVA. Statistical differences were analyzed using Tukey’s Multiple Comparison Test.
	Metric 22:	Reporting of Data	Low	Results were presented in the text for the exposure and the control responses in section 3.1.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported for the DEHP accumulation in the liver.
Additional Comments:	This portion of the evaluation was on the accumulation of DEHP in the liver after 14 days of exposure. ADME was selected as the outcome of interest.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to waterborne di-(2-ethylhexyl) phthalate. <i>General and Comparative Endocrinology</i> 254:22-37.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Neurotoxicology
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5494023

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2: Test Substance Source	High	The source of the DEHP was reported to be from Tokyo Chemical Industry in Japan, and it was verified by GC-MSD.
	Metric 3: Test Substance Purity	High	The DEHP purity was reported to be 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control in which ethanol was used. Ethanol was the reported vehicle solvent in the treatments.
	Metric 5: Negative Control Response	High	The negative control response was reported and adequate for the outcomes of interest. The negative control responses can be found in Figures 2-10.
	Metric 6: Randomized Allocation	Medium	It was reported the fish were randomly allocated into 10 120L tanks with 60L of dechlorinated tap water with 50 fish per exposure group.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The system was reported to be a static renewal system with the test medium renewed every third day for the duration of the study. The stock solution was prepared by dissolving 10mL of DEHP in 90mL of ethanol to get 109.5g/L DEHP. Test concentrations were prepared through serial dilutions with dechlorinated tap water.
	Metric 8: Consistency of Exposure Administration	High	All exposures were in 120L tanks with 50 fish per tank. Each tank had 60L of test solution that was diluted with dechlorinated tap water. All tests were conducted at 24C.
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the exposure concentrations were measured at any point in the study.
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be 14 days. This was adequate to observe a response.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	There were 4 exposure groups. The spacing was adequate to see a biphasic response.
	Metric 12: Testing at or Below Solubility Limit	High	The highest concentration tested was above the water solubility limit of DEHP. However, ethanol was used as a vehicle solvent, and the solvent control results were adequate.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The <i>C. gariepinus</i> were reported to be from the hatchery unit of the Aquaculture Department at the University of Ibadan. They were reported to be fingerlings that were 4 weeks old.
	Metric 14: Acclimatization and Pretreatment Conditions	High	The fish were reported to be acclimated to test conditions for 2 weeks prior to the start of the study.

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<b>Study Citation:</b>	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to waterborne di-(2-ethylhexyl) phthalate. <i>General and Comparative Endocrinology</i> 254:22-37.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Neurotoxicology			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494023			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that there were 50 fish per test chamber. However, there were only 2 replicates for each exposure concentration.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	The fish were held at 24C with a pH of 7 and a DO of 3mg/L. A natural photoperiod was reported to be used, but was not specified. The fish were fed twice daily throughout the experiment. Other properties of the test water were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—the gene expression of acute steroidogenic pathways in the brain and kidneys, as well as hormone levels.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fish were sampled on days 3, 7, and 14, and head kidney and brain samples were harvested and snap frozen in liquid nitrogen and preserved at -80C until processing. Real-time PCR was used for gene expression analysis. Steroid hormone analysis was performed using EIA kits. Immunochemical analysis was performed according to Bradford (1976).	
Domain 6: 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 Presentation and Analysis				
	Metric 21: Statistical Methods	High	All data were presented as +- mean standard deviation and were analyzed using Prism GraphPad 5. Significant differences between the solvent control and exposure groups were performed using one-way ANOVA. Statistical differences were analyzed using Tukey’s Multiple Comparison Test.	
	Metric 22: Reporting of Data	High	Data for the exposure and the control responses were reported in Figures 2-10 and displayed the biphasic response. Table 2 displayed hormone concentrations.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variation was reported in the figures and the tables.	
Additional Comments:	This portion of the evaluation was on the effect of DEHP on gene expression of the steroidogenic pathways of the kidney head and the brain. Several genes in these pathways were measured and a biphasic response was observed. Samples were taken on days 3, 7, and 14. Hormone levels were also assessed. Mechanistic outcomes for neurotoxicity, endocrine toxicity, and cell signaling and function were chosen as the outcomes of interest.			

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<b>Study Citation:</b>	Arukwe, A., Ibor, O. R., Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish ( <i>Clarias gariepinus</i> ) exposed to waterborne di-(2-ethylhexyl) phthalate. <i>General and Comparative Endocrinology</i> 254:22-37.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Clarius gariepinus</i> ; Juvenile
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity-Neurotoxicology
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5494023

Domain	Metric	Rating	Comments
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test performed was at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
	Metric 17: Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**

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

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<b>Study Citation:</b>	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. Journal of Environmental Sciences 46:47-54.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinus carpio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5554274			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The methods section indicated three concentrations of DEHP used in the exposure. There was no further mention or showing of data for concentration specific results. Incubating oocytes to different concentrations seemed irrelevant to the molecular analyses of interest.	
	Metric 12: Testing at or Below Solubility Limit	Low	It is unclear from the text in the methods section whether a solvent was used to get DEHP into solution for the different concentration exposures.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The study reported that gravid adult female common carp were obtained from a local aquatic market in Xiamen. No information was given on what water source they were harvested in. These fish oocytes were removed for use in the exposure study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Study authors did not mention specific pre-treatment conditions for the oocytes other than saying once they were removed from the fish, they were then incubated in Cortland's solution, and the pH was adjusted to 7.4.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Each experimental sample was amplified in triplicate. The Ct values (per reference gene) determined by qPCR were based off of an n=15.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Not much was mentioned on the environmental conditions during the incubation period of oocytes in DEHP concentrations. Oocytes were incubated in Cortland's solution containing streptomycin and penicillin and various nutrients. The cDNA synthesized after the RNA extraction was stored at -20C until use in the qPCR. The qPCR was run under appropriate conditions to get absolute expression value (threshold cycles) results.	
	Metric 17: Outcome Assessment Methodology	High	The methods to extract RNA from the exposed oocytes and then synthesize cDNA from that were adequately described. The primer design was sufficient and the qPCR analyses were adequate to obtain the expression profiles of the reference genes.	
	Metric 18: Consistency of Outcome Assessment	High	There was no information in the study to indicate any differences in molecular analyses among control and treatment groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There was not enough information on environmental conditions throughout the incubation period for the oocytes to say whether there were any confounding variables or differences between treatment groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among treatment groups.	
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	Shi, Y., Lu, J., Wang, Y., Wang, S. (2016). Reference gene validation for quantification of gene expression during final oocyte maturation induced by diethylstilbestrol and di-(2-ethylhexyl)-phthalate in common carp. Journal of Environmental Sciences 46:47-54.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	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)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinus carpio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5554274

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

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

## Overall Quality Determination

**Medium**

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

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<b>Study Citation:</b>	Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp ( <i>Cyprinus carpio</i> ) and antioxidant response by biomarker. <i>Ecotoxicology</i> 23(4):626-632.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinus carpio</i> ; Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 / 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The reported LC50 from this study was 37.9 mg/L for DEHP. The solubility listed in the Final Scope for this compound is 0.27 mg/L.

## Overall Quality Determination

## Medium

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

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<b>Study Citation:</b>	Zhao, X., Gao, Y., Qi, M. (2014). Toxicity of phthalate esters exposure to carp ( <i>Cyprinus carpio</i> ) and antioxidant response by biomarker. <i>Ecotoxicology</i> 23(4):626-632.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinus carpio</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 / 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB Strain; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2298079			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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	The sources were listed.	
	Metric 3: Test Substance Purity	Low	The purity/grade were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both a blank control and 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 Characterization				
	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 a 72 hr period.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures appear to have been administered consistently.	
	Metric 9: Measurement of Test Substance Concentration	Low	Concentrations are reported as nominal.	
	Metric 10: Exposure Duration and Frequency	Medium	Acute exposures were 72-hr for the embryos, which is slightly shorter than the standard 96-hour acute bioassay.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Authors reported conducting exposures at 9 concentrations: 500, 100, 50, 10, 1.5, 0.6, 0.3, 0.06, and 0.01 mg/L.	
	Metric 12: Testing at or Below Solubility Limit	Low	Several concentrations were above estimated solubility limits, including 3-4 treatment concentrations (BBP, DBP) or 7 concentrations (DEHP, DIDP, and DINP). Methanol was utilized to improve solubility.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source and the strain were reported, and the husbandry methods were adequately described.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	An acclimation period was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Each concentration was represented by 20 embryos (1 embryo per well).	
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB Strain; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2298079

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The 24 well plates were described but lacked details on water conditions (or use of embryo rearing medium) and temperature.
	Metric 17: Outcome Assessment Methodology	Low	It was not reported how mortality was determined in the embryos.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and control groups at 72 hr post treatment.
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 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 bioassay.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	There were no details on LC50 estimation methods.
	Metric 22: Reporting of Data	Low	The authors report LC50 values for BBP and DBP (and LC50 not attained for DEHP, DINP, and DIDP). Mortality was not reported for each treatment group or for the controls.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. <i>Aquatic Toxicology</i> 175:286-298.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild Type, long tail; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3350278			
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.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	The 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 the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms varied. It was unclear if replicates were used
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.

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<b>Study Citation:</b>	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. <i>Aquatic Toxicology</i> 175:286-298.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild Type, long tail; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3350278

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods (including any calculations or data transformations) were clearly described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Results from mixtures were also reported.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. <i>Aquatic Toxicology</i> 175:286-298.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild Type, long tail; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3350278			
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.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	The 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 the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was tested.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of organisms varied. It was unclear if replicates were used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	Kinch, C. D., Kurrasch, D. M., Habibi, H. R. (2016). Adverse morphological development in embryonic zebrafish exposed to environmental concentrations of contaminants individually and in mixture. <i>Aquatic Toxicology</i> 175:286-298.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild Type, long tail; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3350278		
Domain	Metric	Rating	Comments
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	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:	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: Results from mixtures were also reported.			
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Cardiovascular			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1: Test Substance Identity	High	Chemicals were identified by CASRN.	
	Metric 2: Test Substance Source	High	Chemicals were purchased from Sigma-Aldrich Beijing.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design	Metric 4: Negative Controls	High	Controls with 0.1% DMSO were used.	
	Metric 5: Negative Control Response	Low	Mortality in controls was approximately 10%.	
	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 test system was not described in any detail, and no further citation was given for preparation of test substances. Methods mention six-well plates with 5 mL solution per well, and six exposure concentrations per chemical. It can be inferred that the exposure was conducted in these well plates, but no details are given.	
	Metric 8: Consistency of Exposure Administration	Low	Details were not reported. It is not stated whether test substances were renewed during the study period or when dosing was performed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and it is not stated whether test solutions were renewed over the course of the exposure. Based on the hydrolysis, photodegradation, and other degradation pathways of the phthalates, this is likely to have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was 7 days; frequency of dosing was not reported, but it can be inferred that no renewal of test substance took place.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Six doses at a wide range of concentrations were chosen for each phthalate.	
	Metric 12: Testing at or Below Solubility Limit	Uninformative	Exposures were well above the solubility limit for DEHP, 3 µg/L.	
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	Low	The source of test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization was not mentioned; embryos were transferred directly to plates at the beginning of the experiment.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Five embryos per concentration were tested in triplicate, for a total of fifteen per concentration.	

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<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Cardiovascular			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and experimental conditions was not reported beyond the mention of six-well plates. Photoperiod, temperature, feeding, and other water quality characteristics were not mentioned.	
Metric 17:	Outcome Assessment Methodology	High	Heart rate was assessed using a recording from a Nikon stereomicroscope.	
Metric 18:	Consistency of Outcome Assessment	High	There was no evidence of outcomes assessed differently across exposure 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 or other non-treatment-related factors across study groups.	
Metric 20:	Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.	
Domain 7: Data Presentation and Analysis				
Metric 21:	Statistical Methods	High	Statistical analysis was performed via T-test in SPSS, with normality confirmed by Kolmogorov-Smirnov.	
Metric 22:	Reporting of Data	High	Outcomes were reported for each exposure group.	
Metric 23:	Explanation of Unexpected Outcomes	High	Unexplained outcomes were not reported.	
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Chemicals were identified by CASRN.	
	Metric 2: Test Substance Source	Low	Chemicals were purchased from Sigma-Aldrich Beijing, but it was not reported if they were analytically verified.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Controls with 0.1% DMSO were used.	
	Metric 5: Negative Control Response	Low	Mortality in controls was approximately 10%.	
	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 test system was not described in any detail, and no further citation was given for preparation of test substances. Methods mention six-well plates with 5 mL solution per well, and six exposure concentrations per chemical. It can be inferred that the exposure was conducted in these well plates, but no details are given.	
	Metric 8: Consistency of Exposure Administration	Low	Details were not reported. It is not stated whether test substances were renewed during the study period or when dosing was performed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and it is not stated whether test solutions were renewed over the course of the exposure. Based on the hydrolysis, photodegradation, and other degradation pathways of the phthalates, this is likely to have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was 7 days; frequency of dosing was not reported, but it can be inferred that no renewal of test substance took place.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Six doses at a wide range of concentrations were chosen for each phthalate.	
	Metric 12: Testing at or Below Solubility Limit	Uninformative	Test concentrations were far above the solubility limit for DEHP (3 µg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization was not mentioned; embryos were transferred directly to plates at the beginning of the experiment.	
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<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Five embryos per concentration were tested in triplicate, for a total of fifteen per concentration. There were 30 fertilized eggs per six-well plate with 5mL of test solution.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and experimental conditions was not reported beyond the mention of six-well plates. Photoperiod, temperature, feeding, and other water quality characteristics were not mentioned.	
	Metric 17: Outcome Assessment Methodology	High	Mortality was assessed via microscopy.	
	Metric 18: Consistency of Outcome Assessment	High	There was no evidence of outcomes assessed differently across exposure 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 or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analysis was performed via T-test in SPSS, with normality confirmed by Kolmogorov-Smirnov.	
	Metric 22: Reporting of Data	High	Outcomes were reported for each exposure group.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Measures of variability were not reported for this outcome (mean only).	
Additional Comments:	This evaluation is for the effect of DEHP on embryo mortality after seven days of exposure. This portion of the evaluation received an unacceptable rating due to the test concentrations being well above the water solubility limit.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Chemicals were identified by CASRN.	
	Metric 2: Test Substance Source	Low	Chemicals were purchased from Sigma-Aldrich Beijing, but it was not reported if they were analytically verified.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Controls with 0.1% DMSO were used.	
	Metric 5: Negative Control Response	Low	Mortality in controls was approximately 10%.	
	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 test system was not described in any detail, and no further citation was given for preparation of test substances. Methods mention six-well plates with 5 mL solution per well, and six exposure concentrations per chemical. It can be inferred that the exposure was conducted in these well plates, but no details are given.	
	Metric 8: Consistency of Exposure Administration	Low	Details were not reported. It is not stated whether test substances were renewed during the study period or when dosing was performed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and it is not stated whether test solutions were renewed over the course of the exposure. Based on the hydrolysis, photodegradation, and other degradation pathways of the phthalates, this is likely to have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was 7 days; frequency of dosing was not reported, but it can be inferred that no renewal of test substance took place.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Six doses at a wide range of concentrations were chosen for each phthalate.	
	Metric 12: Testing at or Below Solubility Limit	Uninformative	Exposure was well above the solubility limit for DEHP, 3 µg/L.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization was not mentioned; embryos were transferred directly to plates at the beginning of the experiment.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Five embryos per concentration were tested in triplicate, for a total of fifteen per concentration. There were 30 fertilized eggs per six-well plate with 5mL of test solution.	
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and experimental conditions was not reported beyond the mention of six-well plates. Photoperiod, temperature, feeding, and other water quality characteristics were not mentioned.	
	Metric 17: Outcome Assessment Methodology	Uninformative	The reported outcome was "malformations", but only partial examples of what the authors mean by this are given in the text. Bent spine and pericardial edema are given as examples, but the text says that "At 96 hpf stage, all zebrafish larvae exposed to PAEs showed different defects" without elaboration. The rate of deformed fish is given in Fig. 5, but no detail is given on which deformations are counted and what methodology was used to identify deformed fish. Further, it seems for DBP and BBP that dead fish were counted in this measurement, regardless of when in the study they died. Exposures with 100% mortality are shown in the table of malformations, at the same 7-d outcome assessment period. This lack of clarity renders this outcome unacceptable.	
	Metric 18: Consistency of Outcome Assessment	High	There was no evidence of outcomes assessed differently across exposure 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 or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analysis was performed via T-test in SPSS, with normality confirmed by Kolmogorov-Smirnov.	
	Metric 22: Reporting of Data	High	Outcomes were reported for each exposure group.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Measures of variability were not reported for this outcome (mean only).	
Additional Comments:	This evaluation is for the effect of DEHP on embryo development after seven days of exposure. This portion of the study received an unacceptable rating because the outcome assessment was unclear (See Metric 17 for detailed description) and because the test concentrations were well above the water solubility limit for DEHP.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemicals were identified by CASRN.
	Metric 2:	Test Substance Source	Low	Chemicals were purchased from Sigma-Aldrich Beijing, but it was not reported if they were analytically verified.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls with 0.1% DMSO were used.
	Metric 5:	Negative Control Response	Low	Mortality in controls was approximately 10%.
	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 test system was not described in any detail, and no further citation was given for preparation of test substances. Methods mention six-well plates with 5 mL solution per well, and six exposure concentrations per chemical. It can be inferred that the exposure was conducted in these well plates, but no details are given.
	Metric 8:	Consistency of Exposure Administration	Low	
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and it is not stated whether test solutions were renewed over the course of the exposure. Based on the hydrolysis, photodegradation, and other degradation pathways of the phthalates, this is likely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was 7 days; frequency of dosing was not reported, but it can be inferred that no renewal of test substance took place.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Six doses at a wide range of concentrations were chosen for each phthalate.
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	Exposures were well above the solubility limit for DEHP, 3 µg/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization was not mentioned; embryos were transferred directly to plates at the beginning of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Five embryos per concentration were tested in triplicate, for a total of fifteen per concentration. There were 30 fertilized eggs per six-well plate with 5mL of test solution.
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and experimental conditions was not reported beyond the mention of six-well plates. Photoperiod, temperature, feeding, and other water quality characteristics were not mentioned.
	Metric 17:	Outcome Assessment Methodology	High	Movement rate (behavior) was assessed using a recording from a Nikon stereomicroscope.
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence of outcomes assessed differently across exposure 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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed via T-test in SPSS, with normality confirmed by Kolmogorov-Smirnov.
	Metric 22:	Reporting of Data	High	Outcomes were reported for each exposure group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexplained outcomes were not reported.
Additional Comments:	This evaluation is for behavior outcomes in embryos after exposure to DEHP. These were measured in terms of movement after the exposure. These assessments were conducted at 24hpf. This evaluation received an unacceptable rating due to exposure concentrations being well above the water solubility limit.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemicals were identified by CASRN.
	Metric 2:	Test Substance Source	Low	Chemicals were purchased from Sigma-Aldrich Beijing, but it was not reported if they were analytically verified.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls with 0.1% DMSO were used.
	Metric 5:	Negative Control Response	Low	Controls mortality was approximately 10%.
	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 test system was not described in any detail, and no further citation was given for preparation of test substances. Methods mention six-well plates with 5 mL solution per well, and six exposure concentrations per chemical. It can be inferred that the exposure was conducted in these well plates, but no details are given.
	Metric 8:	Consistency of Exposure Administration	Low	
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and it is not stated whether test solutions were renewed over the course of the exposure. Based on the hydrolysis, photodegradation, and other degradation pathways of the phthalates, this is likely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was 7 days; frequency of dosing was not reported, but it can be inferred that no renewal of test substance took place.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was used for the gene expression study.
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	The exposure was well above the solubility limit for DEHP, 3 µg/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization was not mentioned; embryos were transferred directly to plates at the beginning of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Only one test concentration was used for the gene expression test, meaning that 5 embryos were used.
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Pu, S. Y., Hamid, N., Ren, Y. W., Pei, D. S. (2020). Effects of phthalate acid esters on zebrafish larvae: Development and skeletal morphogenesis. Chemosphere 246:125808.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5932877			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and experimental conditions was not reported beyond the mention of six-well plates. Photoperiod, temperature, feeding, and other water quality characteristics were not mentioned.
	Metric 17:	Outcome Assessment Methodology	High	Gene expression was quantified using qRT-PCR.
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence of outcomes assessed differently across exposure 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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not performed, and no data allowing analysis was provided for the gene expression tests.
	Metric 22:	Reporting of Data	High	Outcomes were reported for each exposure group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not reported for this outcome (mean only).
Additional Comments:	This evaluation was for the gene expression assessment performed on zebrafish embryos after exposure to DEHP. This portion of the evaluation received an unacceptable rating due to lack of statistical analysis and because the test concentrations of DEHP were well above the water solubility limit.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported to be 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 and appropriate concurrent negative 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 embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was not reported in great detail, nor was the preparation of the test solutions.	
	Metric 8: Consistency of Exposure Administration	Low	Little details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be up 120hpf. Samples were also taken at 24hpf. This was adequate to observe the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	It was reported there were five test concentrations that increased by a factor of 10. There were two replicates per test concentration.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	It was reported that zebrafish were raised at Oregon State University Sinnhuber Aquatic Laboratory in Corvallis, OR. It is unclear if the zebrafish were obtained from another lab and raised solely for this study, or if they were cultured at the facility long term.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if zebrafish were acclimated at any point.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 32 total embryos exposed to each test concentration split into two replicates.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Little details were reported regarding organism housing or environmental conditions.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-mortality at both 24hpf and 120hpf as reported in the supplementary material.
	Metric 18:	Consistency of Outcome Assessment	Medium	Embryos were evaluated for mortality at 24hpf and 120hpf during the exposure. Details regarding how mortality was evaluated were not reported in detail.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Details regarding environmental conditions before and during the study were limited.
	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 analysis is reported in Section 3 "Theory and calculation."
	Metric 22:	Reporting of Data	Uninformative	This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.
<b>Additional Comments:</b>	This evaluation is for the effect of DEHP on zebrafish embryo mortality. Data is reported in Table S1.This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported to be 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 and appropriate concurrent negative 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 embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was not reported in great detail, nor was the preparation of the test solutions.	
	Metric 8: Consistency of Exposure Administration	Low	Little details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be up 120hpf. Samples were also taken at 24hpf. This was adequate to observe the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	It was reported there were five test concentrations that increased by a factor of 10. There were two replicates per test concentration.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	It was reported that zebrafish were raised at Oregon State University Sinnhuber Aquatic Laboratory in Corvallis, OR. It is unclear if the zebrafish were obtained from another lab and raised solely for this study, or if they were cultured at the facility long term.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if zebrafish were acclimated at any point.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 32 total embryos exposed to each test concentration split into two replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Little details were reported regarding organism housing or environmental conditions.	
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<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest- changes in behavior in terms of spontaneous movement and change in movement at light-to-dark transition time point. Embryos were evaluated for changes in behavior were assessed using larval photomotor response assay using Viewpoint Zebrafish.
	Metric 18:	Consistency of Outcome Assessment	High	
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Details regarding environmental conditions before and during the study were limited.
	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 analysis is reported in Section 3 "Theory and calculation."
	Metric 22:	Reporting of Data	Uninformative	This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.
Additional Comments:	This evaluation is for the effect of DEHP on zebrafish embryo behavior. Data is reported in Table S1.This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported to be 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 and appropriate concurrent negative 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 embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was not reported in great detail, nor was the preparation of the test solutions.	
	Metric 8: Consistency of Exposure Administration	Low	Little details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be up 120hpf. Samples were also taken at 24hpf. This was adequate to observe the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	It was reported there were five test concentrations that increased by a factor of 10. There were two replicates per test concentration.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	It was reported that zebrafish were raised at Oregon State University Sinnhuber Aquatic Laboratory in Corvallis, OR. It is unclear if the zebrafish were obtained from another lab and raised solely for this study, or if they were cultured at the facility long term.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if zebrafish were acclimated at any point.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 32 total embryos exposed to each test concentration split into two replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Little details were reported regarding organism housing or environmental conditions.	
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<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-no response to touch at 120hpf as reported in the supplementary material.	
	Metric 18: Consistency of Outcome Assessment	Medium	Embryos were evaluated for touch response at 120hpf during the exposure. Details regarding how mobility was evaluated were not reported in detail.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Details regarding environmental conditions before and during the study were limited.	
	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 analysis is reported in Section 3 "Theory and calculation."	
	Metric 22: Reporting of Data	Uninformative	This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.	
Additional Comments:	This evaluation is for the effect of DEHP on zebrafish embryo mobility/immobilization. Data is reported in Table S1.This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported to be 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 and appropriate concurrent negative 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 embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was not reported in great detail, nor was the preparation of the test solutions.	
	Metric 8: Consistency of Exposure Administration	Low	Little details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be up 120hpf. Samples were also taken at 24hpf. This was adequate to observe the outcomes of interest.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	It was reported there were five test concentrations that increased by a factor of 10. There were two replicates per test concentration.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	It was reported that zebrafish were raised at Oregon State University Sinnhuber Aquatic Laboratory in Corvallis, OR. It is unclear if the zebrafish were obtained from another lab and raised solely for this study, or if they were cultured at the facility long term.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if zebrafish were acclimated at any point.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 32 total embryos exposed to each test concentration split into two replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Little details were reported regarding organism housing or environmental conditions.	
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<b>Study Citation:</b>	Thomas, D. G., Shankaran, H., Truong, L., Tanguay, R. L., Waters, K. M. (2019). Time-dependent behavioral data from zebrafish reveals novel signatures of chemical toxicity using point of departure analysis. Computational Toxicology 9:50-60.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8635978			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest- changes in development/growth reported as changes in morphology. All morphology endpoints assessed are reported in Table 1.
	Metric 18:	Consistency of Outcome Assessment	Medium	Embryos were evaluated for changes in morphology at 24hpf and 120hpf. The exact methods for assessing morphology were not reported in great detail.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Details regarding environmental conditions before and during the study were limited.
	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 analysis is reported in Section 3 "Theory and calculation."
	Metric 22:	Reporting of Data	Uninformative	This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.
Additional Comments:	This evaluation is for the effect of DEHP on zebrafish embryo morphology. Data is reported in Table S1.This study is a reanalysis or replication (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) of HEROID 8591199; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Further, the BMD10 values in Table S1 cannot follow from the LEL values reported in Table S5; endpoints with LELs at the lowest concentration tested are shown with "No effect" BMD10s. These inconsistencies render this pair of studies unacceptable for use.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8591199			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported. 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.	
	Metric 5: Negative Control Response	High	The negative control response for mortality is reported in the supplemental material under Supplementary Figure 1.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Embryos were statically exposed to the test chemicals in 96-well plate. Each well had one 6hpf embryo. Test concentrations were prepared using a 10-fold serial dilution. DMSO was used as a solvent in the embryo medium.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were conducted consistently across study groups. All exposures were conducted statically in 96-well plates under similar environmental conditions.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test substance was measured at any point in the study, nor were any values reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure administration was reported to be 120hpf. Embryos were also sampled at 24hpf for assessment of some parameters. These time points were adequate for a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups, each separated by an order of magnitude. This was adequate to observe a response and to compare responses between chemicals.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	It was reported that tropical 5D wild-type zebrafish were housed in a density of 1000 fish per 100 gallon tank at Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR. However, it is unclear if the fish were cultured here or obtained from another source. Embryos were used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Test conditions and holding conditions appeared similar.	

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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8591199			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There was one embryo placed in each well, and there were 32 replicates for each test concentration.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Adult fish were housed at 28C with 1000 fish in 100 gallon tanks. Fish were housed in reverse osmosis water supplemented with Instant Ocean, but other water characteristics were not reported. A 14L:10D photoperiod was used. The feeding regimen was not reported. Embryos were tested at 28C in standard embryo media, but characteristics of this were 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	High	Outcomes appeared to be assessed consistently across study groups using PRAT.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Embryos were held at a similar temperature to holding 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 analysis was performed using code developed in R. Details of this can be found in the Analysis section.	
	Metric 22: Reporting of Data	Uninformative	This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HEROID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.	
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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	8591199		

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the effect of DEHP on embryo mortality after exposure to five test concentrations increasing by 10 fold. Embryos were exposed starting at 6hpf to 120hpf. Mortality data can be found in Supplemental Figure 1. This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HERO ID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.		

**Overall Quality Determination****Uninformative**



<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8591199			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	Low	The source of the test substance was not reported. 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.
	Metric 5:	Negative Control Response	High	The negative control responses for several development and growth outcomes are reported in the supplemental material under Supplementary Figure 1.
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Embryos were statically exposed to the test chemicals in 96-well plate. Each well had one 6hpf embryo. Test concentrations were prepared using a 10-fold serial dilution. DMSO was used as a solvent in the embryo medium.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were conducted consistently across study groups. All exposures were conducted statically in 96-well plates under similar environmental conditions.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test substance was measured at any point in the study, nor were any values reported.
	Metric 10:	Exposure Duration and Frequency	High	The exposure administration was reported to be 120hpf. Embryos were also sampled at 24hpf for assessment of some parameters. These time points were adequate for a response.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups, each separated by an order of magnitude. This was adequate to observe a response and to compare responses between chemicals.
	Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	It was reported that tropical 5D wild-type zebrafish were housed in a density of 1000 fish per 100 gallon tank at Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR. However, it is unclear if the fish were cultured here or obtained from another source. Embryos were used for this study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test conditions and holding conditions appeared similar.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There was one embryo placed in each well, and there were 32 replicates for each test concentration.

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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	8591199		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Adult fish were housed at 28C with 1000 fish in 100 gallon tanks. Fish were housed in reverse osmosis water supplemented with Instant Ocean, but other water characteristics were not reported. A 14L:10D photoperiod was used. The feeding regimen was not reported. Embryos were tested at 28C in standard embryo media, but characteristics of this were not reported.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-several development and growth parameters.
Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups using PRAT.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	Embryos were held at a similar temperature to holding 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 analysis was performed using code developed in R. Details of this can be found in the Analysis section.
Metric 22:	Reporting of Data	Uninformative	This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HERO ID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.
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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. <i>Toxicological Sciences</i> 137(1):212-233.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	8591199

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the effect of DEHP on embryo growth and development after exposure to five test concentrations increasing by 10 fold. Embryos were exposed starting at 6hpf to 120hpf. Development and growth data can be found in Supplemental Figure 1. These parameters include developmental delay and developmental changes in the notochord, yolk sac edema, body axis, eye defect, snout, jaw, otic vesicle, pericardial edema, brain, somite, pectoral fin, caudal fin, pigment, circulation, truncated body, swim bladder, and bent tail. This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HERO ID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.		

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	8591199			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported. 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.	
	Metric 5: Negative Control Response	High	The negative control responses for behavioral outcomes are reported in the supplemental material under Supplementary Figure 1.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Embryos were statically exposed to the test chemicals in 96-well plate. Each well had one 6hpf embryo. Test concentrations were prepared using a 10-fold serial dilution. DMSO was used as a solvent in the embryo medium.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were conducted consistently across study groups. All exposures were conducted statically in 96-well plates under similar environmental conditions.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test substance was measured at any point in the study, nor were any values reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure administration was reported to be 120hpf. Embryos were also sampled at 24hpf for assessment of some parameters. These time points were adequate for a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups, each separated by an order of magnitude. This was adequate to observe a response and to compare responses between chemicals.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	It was reported that tropical 5D wild-type zebrafish were housed in a density of 1000 fish per 100 gallon tank at Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR. However, it is unclear if the fish were cultured here or obtained from another source. Embryos were used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Test conditions and holding conditions appeared similar.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There was one embryo placed in each well, and there were 32 replicates for each test concentration.	

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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	8591199		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Adult fish were housed at 28C with 1000 fish in 100 gallon tanks. Fish were housed in reverse osmosis water supplemented with Instant Ocean, but other water characteristics were not reported. A 14L:10D photoperiod was used. The feeding regimen was not reported. Embryos were tested at 28C in standard embryo media, but characteristics of this were not reported.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in behavior in terms of spontaneous movement and touch response.
Metric 18:	Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups using PRAT.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	Embryos were held at a similar temperature to holding 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 analysis was performed using code developed in R. Details of this can be found in the Analysis section.
Metric 22:	Reporting of Data	Uninformative	This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HEROID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.
Additional Comments:	This evaluation is for the effect of DEHP on behavior after exposure to five test concentrations increasing by 10 fold. Embryos were exposed starting at 6hpf to 120hpf. Behavioral data can be found in Supplemental Figure 1. Behavioral responses assessed include spontaneous movement and touch response. This study was reanalyzed or replicated (it is unclear from the text whether the experiment was repeated or whether the same data was reanalyzed) in HEROID 8635978; but the results for the same chemicals vary by up to 5 orders of magnitude for the same experimental results between the two studies. Several of the phthalates exhibited no observable effect at any concentration studied in this study, but LELs at concentrations 10,000 times lower in 8635978 using identical methodology. These inconsistencies render this pair of studies unacceptable for use.		

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<b>Study Citation:</b>	Truong, L., Reif, D. M., Mary, L. S., Geier, M. C., Truong, H. D., Tanguay, R. L. (2014). Multidimensional in vivo hazard assessment using zebrafish. Toxicological Sciences 137(1):212-233.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Tropical 5D wild-type; Embryo		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	8591199		
Domain	Metric	Rating	Comments
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

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

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	The environmental conditions were not sufficiently reported to evaluate if they were adequate. Some parameters were reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	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	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	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**



<b>Study Citation:</b>	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylhexyl) phthalate impairs spermatogenesis in zebrafish ( <i>Danio rerio</i> ). <i>Reproductive Biology</i> 13(3):195-202.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2000753			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported and reasonable for 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	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations.	
	Metric 8: Consistency of Exposure Administration	Low	The study provided few details on the exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	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	Only two levels were tested, but an adequate response was observed.	
	Metric 12: Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit. The second concentration, 20 µg/L, exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were adequate numbers to allow subsampling, but replicates were not used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate. Some parameters were reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
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<b>Study Citation:</b>	Corradetti, B., Stronati, A., Tosti, L., Manicardi, G., Carnevali, O., Bizzaro, D. (2013). Bis-(2-ethylexhyl) phthalate impairs spermatogenesis in zebrafish ( <i>Danio rerio</i> ). <i>Reproductive Biology</i> 13(3):195-202.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2000753

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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	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**

<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult			
<b>Health Outcome:</b>	Gastrointestinal			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043619			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemical descriptors were not provided.	
Metric 2:	Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.	
Metric 3:	Test Substance Purity	High	The purity was given as "pure." A search on the Sigma Aldrich site for the given stock number shows it was analytical standard.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A negative control was included.	
Metric 5:	Negative Control Response	High	The response of the negative control was acceptable.	
Metric 6:	Randomized Allocation	Medium	A randomized allocation method was mentioned in the supplementary methods.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	A flow-through system was detailed in the supplementary methods.	
Metric 8:	Consistency of Exposure Administration	High	The DEHP was administered in the feed; details of the preparation were given in the supplemental methods.	
Metric 9:	Measurement of Test Substance Concentration	High	The concentration was measured by GC/MS (n=3).	
Metric 10:	Exposure Duration and Frequency	High	The organisms were fed 2x daily for 60 days.	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	The study only had one dose (3 mg/kg feed), and it was not intended to examine a dose-response effect.	
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).	
Metric 14:	Acclimatization and Pretreatment Conditions	High	An acclimatization period of 1 week was noted in the supplemental material.	
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<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult			
<b>Health Outcome:</b>	Gastrointestinal			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043619			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The main experimental design included: 6 fish per tank, 10 replicate tanks per treatment, 60 fish per group, and 30 tanks total. Samples for histology included: stained sections of distal intestine from both treatment groups and the control group (n = 8 for each treatment).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal husbandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.
	Metric 17:	Outcome Assessment Methodology	High	Gut histology was performed at the end of the experiment.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across treatments.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Authors reported that stained sections of the distal intestine from both treatment groups and the control group were evaluated for histological alterations. That is, they were histological observations. Statistical analysis was not necessary.
	Metric 22:	Reporting of Data	High	Data was reported for all groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes in histological gut analysis.
Additional Comments:	The objective of this study was to determine how DEHP in the diet may exacerbate mechanisms associated with weight gain that occurs with overfeeding. Zebrafish were orally exposed to overfeeding and overfeeding with DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured by weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI), histological examination of the intestine, and changes in gene expression in the intestine (using qPCR and RNAseq). This form was used to evaluate data on the histological examination of the intestine.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043619			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemical descriptors were not provided.
	Metric 2:	Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.
	Metric 3:	Test Substance Purity	High	The purity given as "pure." A search on the Sigma Aldrich site for the given stock number shows it was analytical standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was included.
	Metric 5:	Negative Control Response	High	The responses of the negative controls were acceptable.
	Metric 6:	Randomized Allocation	Medium	A randomized allocation method was mentioned in the supplementary methods.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	A flow-through system was detailed in the supplementary methods.
	Metric 8:	Consistency of Exposure Administration	High	DEHP was administered in the feed; details of the preparation were given in the supplemental methods.
	Metric 9:	Measurement of Test Substance Concentration	High	The concentration was measured by GC/MS (n=3).
	Metric 10:	Exposure Duration and Frequency	High	The organisms were fed 2x daily for 60 days.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	The study only had one dose (3 mg/kg feed), and it was not intended to examine a dose-response effect.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An acclimatization period of 1 week was noted in the supplemental material.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The main experimental design was as follows: 6 fish per tank, 10 replicate tanks per treatment, 60 fish per group, and 30 tanks total. RNAseq samples included: control group n=4, overfed group n = 3, overfed + DEHP group n = 5 selected from multiple tanks within each treatment.
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<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5043619		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal husbandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (qPCR of liver and intestine tissues and RNAseq of intestine tissues) were reported and adequate for the intended outcome of interest (gene expression changes related to digestion in the intestine and liver).
Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across treatments.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups in environmental conditions.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods (ANOVA, Kruskal-Wallis tests) were detailed in the supplemental information.
Metric 22:	Reporting of Data	High	The data was reported for all groups.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes in genetic analysis.
Additional Comments:	The objective of this study was to determine how DEHP in the diet may exacerbate mechanisms associated with weight gain that occurs with overfeeding. Zebrafish were orally exposed to overfeeding and overfeeding with DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured by weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI), histological examination of the intestine, and changes in gene expression in the intestine (using qPCR and RNAseq). This form was used to evaluate data on gene expression.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043619			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by nomenclature only. CASRN, structure and/or other chemical descriptors were not provided.	
	Metric 2: Test Substance Source	Low	The source was reported (Sigma Aldrich, Catalog No. 36735), but the DEHP was not analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was given as "pure." A search on the Sigma Aldrich site for the given stock number shows it was an analytical standard.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was included.	
	Metric 5: Negative Control Response	High	The responses of the negative controls were acceptable.	
	Metric 6: Randomized Allocation	Medium	Randomized allocation was mentioned in the supplementary methods.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation (DEHP stock solution and feed treatment with DEHP) was described in detail in the supplemental material and methods.	
	Metric 8: Consistency of Exposure Administration	High	DEHP was administered in feed and administered consistently across treatment groups; details were provided in the supplemental methods.	
	Metric 9: Measurement of Test Substance Concentration	High	Concentrations were measured by GC/MS (n=3).	
	Metric 10: Exposure Duration and Frequency	High	The organisms were fed 2x daily for 60 days.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	The study only had one dose (3 mg/kg feed) and was not intended to examine a dose-response effect.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild type (AB Strain) male and female zebrafish were obtained from the University of Florida (UF) Animal Care Services maintained at the Aquatic Toxicology Laboratory in the Center for Environmental and Human Toxicology (UF).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Acclimatization for 1 week was noted in the supplemental material, and the pre-treatment culture conditions were the same.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 6 fish per tank, 10 replicate tanks per treatment, 60 fish per group, and 30 tanks total.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Buerger, A. N., Schmidt, J., Chase, A., Paixao, C., Patel, T. N., Brumback, B. A., Kane, A. S., Martyniuk, C. J., Bisesi, J. H. (2019). Examining the responses of the zebrafish ( <i>Danio rerio</i> ) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. <i>Environmental Pollution</i> 245(Elsevier):1086-1094.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; Wild type (AB strain); Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043619			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	The test conditions were acceptable for the husbandry of zebrafish. All animal husbandry and experiments followed the UF Institutional Animal Care and Use Committee Protocol number 201408537.	
	Metric 17: Outcome Assessment Methodology	High	Tank mass was measured weekly; individual masses were measured 3x. Gonads and livers were weighed at the end of the experiment to calculate gonadosomatic index (GSI) and hepatosomatic index (HSI).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across treatments.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups in environmental conditions.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods (ANOVA, Kruskal-Wallis tests) were detailed in the supplemental information.	
	Metric 22: Reporting of Data	High	Data was reported for all groups.	
	Metric 23: Explanation of Unexpected Outcomes	High	The expected outcome was an increase in weight gain among DEHP-treated fish in the "overfed" category, but there was no difference noted. The given explanation involving genes for lipid metabolism only partially explained this discrepancy, & the authors hypothesized with limited evidence that a longer exposure would lead to the expected outcome.	
Additional Comments:	The objective of this study was to determine how DEHP in the diet may exacerbate mechanisms associated with weight gain that occurs with overfeeding. Zebrafish were orally exposed to overfeeding and overfeeding with DEHP for 60 days to investigate the exacerbation of DEHP on obesity measured by weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI), histological examination of the intestine, and changes in gene expression in the intestine (using qPCR and RNSseq). This form was used to evaluate weight gain, body mass index, gonadosomatic index (GSI), hepatosomatic index (HSI) under the health outcome of growth/development.			
Overall Quality Determination		High		



<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue number for the chemical. The correct DEHP CASRN is 117-81-7.	
Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.	
Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).	
Metric 5:	Negative Control Response	High	This form was used to evaluate NOT the direct effects of DEHP exposure on male zebrafish, but the indirect effects of fraternal exposure on the F1 generation larvae (off-spring growth/development): hatching rate and body weight.	
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the mating pairs (8 male zebrafish per group were randomly collected for mating with wild type females), and they reported that 100 fertilized eggs were randomly collected and cultured until 5 dpf.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.	
Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.	
Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.	
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture). The fertilized eggs used for the growth/development effects were produced by crossing exposed males with wild-type females.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, adult male zebrafish were acclimatized in 20 L tanks for 1 week.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers were reported as follows: Parental male fish: 8 male zebrafish from each experimental group; 3 replicates per group. This form is to evaluate the offspring/F1 data on growth/development: 100 fertilized eggs were randomly collected per group and cultured until 5 dpf. It is unclear how many parental replicate tanks are represented by the 100 fertilized eggs.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (crossing of DEHP exposed and control male fish with wild-type female fish and collection and culture of fertilized eggs until 5 dpf, counting of hatched larvae, and weighing larvae) were reported and adequate for the outcome of interest (DEHP effects on F1 generation following exposure of parental male fish).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5497528

Domain	Metric	Rating	Comments
	Metric 21: Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean $\pm$ standard error (SEM).
	Metric 22: Reporting of Data	High	Data were reported for all treatment and control groups (Figure 1B).
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

**Additional Comments:** The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentrations by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, and DNA methylation (global and site-specific) and mRNA changes of genes involved in reproduction. This form was used to evaluate the data reported for the F1 generation larvae (offspring growth/development): hatching rate and body weight.

**Overall Quality Determination****High**

<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5497528		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue number for the chemical. The correct DEHP CASRN is 117-81-7.
Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).
Metric 5:	Negative Control Response	High	Fecundity, fertilization success, and histological examination of male gonads were assessed. The biological responses of the solvent control were reported and adequate (Figures 1A, 3, 4, & S1).
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the various health outcomes pertaining to this form (sections 2.4, 2.6, & 2.7).
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.
Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.
Domain 4: Test Organism			
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers and the replicates were reported as follows: Study Total: 16 male fish per tank, 3 replicates per group, and 4 treatment groups (0, 10, 33, and 100 mg/L DEHP) = 48 male fish per group = 192 male fish total. Reproductive capabilities (fecundity and fertilization success): 8 male zebrafish from each experimental group. Light microscopy: 9 fish randomly collected from three tanks in each group. Electron microscopy: 3 fish randomly collected from 3 tanks in control and 100 mg/L DEHPexposure groups.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodologies [fecundity (number of eggs produced per female), fertilization success (number of fertilized eggs), light and electron microscopic examination of gonads] were reported and adequate for the outcome of interest (DEHP effects on reproduction and gonad integrity).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Fecundity, fertilization success, and gonad staging by microscopy: Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean ± standard error (SEM).	
	Metric 22: Reporting of Data	High	Data were reported for all treatment and control groups (Figures 1A, 3, 4, & S1).	
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric		Rating	Comments
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentrations by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, and DNA methylation (global and site-specific) and mRNA changes of genes involved in reproduction. This form was used to evaluate the data reported for male reproductive effects: Fecundity, fertilization success, and histological examination of male gonads (light and electron microscopy).			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5497528		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; instead, this number is the catalogue number for the chemical. The correct DEHP CASRN is 117-81-7.
Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).
Metric 5:	Negative Control Response	High	Plasma reproductive hormone levels (testosterone and 17beta-estradiol) were assessed. The biological responses of the solvent control were reported and adequate.
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the outcome pertaining to this form (reproductive hormones, sections 2.5).
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study, (Wang et al 2013) as well as being environmentally relevant.
Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.
Domain 4: Test Organism			
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers and replicates are as follows: Study Total: 16 male fish per tank, 3 replicates per group, and 4 treatment groups (0, 10, 33, and 100 mg/L DEHP) = 48 male fish per group = 192 male fish total. Reproductive hormone levels (testosterone and 17beta-estradiol): 4 fish per tank wereconsidered as one replicate. There were 3 replicates per group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (collection of blood, extraction of plasma, and use of the competitive ELISA) were reported and adequate for the outcome of interest (DEHP effects on the concentration of reproductive hormones and its reproductive outcomes).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean ± standard error (SEM).	
	Metric 22: Reporting of Data	High	Data were reported for all treatment and control groups (Figure 2).	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5497528

Domain	Metric	Rating	Comments
Additional Comments:	The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentrations by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, DNA methylation (global and site-specific), and mRNA changes of genes involved in reproduction. This form was used to evaluate the following mechanistic data: plasma reproductive hormone levels (testosterone and 17beta-estradiol).		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5497528		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; this is instead the catalogue number for the chemical. The correct DEHP CASRN is 117-81-7.
Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).
Metric 5:	Negative Control Response	High	This form was used to evaluate NOT the direct effects of DEHP exposure on male zebrafish, but the indirect effects of fraternal exposure on the F1 generation larvae survival rate.
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the mating pairs (8 male zebrafish per group were randomly collected for mating with wild type females), and they reported that 100 fertilized eggs were randomly collected and cultured until 5 dpf.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.
Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5497528		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture). The fertilized eggs used for the survival rate effects were produced by crossing exposed males with wild-type females.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, adult male zebrafish were acclimatized in 20 L tanks for 1 week.
Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers used were as follows: Parental male fish: 8 male zebrafish from each experimental group; 3 replicates per group. This form is to evaluate the offspring/F1 survival rate: 100 fertilized eggs were randomly collected per group and cultured until 5 dpf. It is unclear how many parental replicate tanks are represented by the 100 fertilized eggs.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (crossing of DEHP exposed and control male fish with wild-type female fish and collection and culture of fertilized eggs until 5 dpf, taking counts of larvae that survived) were reported and adequate for the outcome of interest (DEHP effects on F1 generation following exposure of parental male fish).
Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean $\pm$ standard error (SEM).
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	High	Data were reported for all treatment and control groups (Figure 1B).
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
<b>Additional Comments:</b>	The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentrations by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, and DNA methylation (global and site-specific) and mRNA changes of genes involved in reproduction. This form was used to evaluate the data reported for the F1 generation larvae (offspring growth/development): survival rate.			
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Epigenetics-Receptor binding/ regulation of receptor activity		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5497528		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The authors identified the chemical by name only [Di-(2-ethylhexyl) phthalate (DEHP)]. The CASRN they reported (ALR-09 7N) is incorrect; instead, this number is the catalogue number for the chemical. The correct DEHP CASRN is 117-81-7.
Metric 2:	Test Substance Source	Low	The source was identified (AccuStandard, New Haven, CT, USA), but the chemical was not analytically verified.
Metric 3:	Test Substance Purity	High	The purity of DEHP was reported as 99.6%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A solvent control was used (0.001% (v/v) DMSO).
Metric 5:	Negative Control Response	High	DNA methylation (global and site-specific) and gene expression (cyp17a1, hsd17b3 and cyp19a1a) in male gonads were assessed. The biological responses of the solvent control were reported and adequate (Figures 5, 6, & 7).
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of zebrafish to the DEHP exposure groups (section 2.3). They only reported random assignment of the various fish per replicate to examine the various health outcomes evaluated in this form (sections 2.7 & 2.9).
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Authors reported the exposure concentrations and the use of solvent (DMSO). They indicated that the exposure was in a semi-static system. They also reported that solutions were replaced (50% daily, 100% weekly) with fresh water containing assigned DEHP concentrations. However, authors did not report how the stock/exposure solutions were prepared.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence to indicate that exposure administration was not consistent across treatment groups.
Metric 9:	Measurement of Test Substance Concentration	Low	The test substance was not analytically verified at the beginning, during, or end of the study.
Metric 10:	Exposure Duration and Frequency	High	A 3-month exposure was appropriate for the study type.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure groups and spacing (control (0.001% DMSO), 10, 33, and 100 ug/L DEHP) were appropriate for the purpose of the study. The authors reported that the nominal exposure concentrations were taken from a previous study (Wang et al 2013) as well as being environmentally relevant.
Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit of DEHP is 270 ug/L. Not only were the exposure concentrations below this limit, but the authors also used 0.001% DMSO to aid dissolve DEHP.
Domain 4: Test Organism			

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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Epigenetics-Receptor binding/ regulation of receptor activity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5497528			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	The test organisms (male zebrafish, AB strain) were adequately described, but the authors did not explicitly clarify the source (e.g., laboratory culture).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Before DEHP exposure, zebrafish were acclimatized in 20 L tanks for 1 week.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers were reported as follows: Study Total: 16 male fish per tank, 3 replicates per group, and 4 treatment groups (0, 10, 33, and 100 mg/L DEHP) = 48 male fish per group = 192 male fish total. DNA methylation (global and site-specific) and gene expression (mRNA changes in cyp17a1, cyp19a1a, and hsd17b3): 2 fish per tank were randomly collected as one replicate, and there were 3 replicates per group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The authors reported: "zebrafish study was approved by the Institutional Animal Care and Use Committee of Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences (Approval ID: ZKCQY0168); experiments were performed according to "Guide for the Care and Use of Laboratory Animals" (Eighth Edition, 2011. ILARCLS, National Research Council, Washington, D.C.)." In addition, authors reported environmental conditions and feeding regimen were appropriate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodologies (DNA methylation (global and site-specific) and gene expression (mRNA changes in cyp17a1, cyp19a1a, and hsd17b3)) were reported and adequate for the outcome of interest (DEHP effects on DNA methylation and genes involved in reproductive capabilities).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Based on information reported by authors, there were no limitations that would result in a substantial impact on results.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were done using one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons tests using SPSS 13.0 software (SPSS, Chicago, IL, USA). P values < 0.05 were considered statistically significant. All data are shown as the mean ± standard error (SEM).	
	Metric 22: Reporting of Data	High	Data were reported for all treatment and control groups (Figures 5, 6, & 7).	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
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<b>Study Citation:</b>	Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. Environmental Pollution 237:1050-1061.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Danio rerio</i> ; AB strain; Adult
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Epigenetics-Receptor binding/ regulation of receptor activity
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5497528

Domain	Metric	Rating	Comments
Additional Comments:	The goal of this study was to investigate the chronic impacts of a 3-month DEHP exposure on male reproduction with environmentally relevant concentrations by examining the effect of DEHP on male reproductive capabilities, offspring growth/development, plasma reproductive hormone levels, DNA methylation (global and site-specific), and mRNA changes of genes involved in reproduction. This form was used to evaluate the following mechanistic data: DNA methylation (global and site-specific) and gene expression of cyp17a1, cyp19a1a, and hsd17b3.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071151			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not reported.	
Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).	
Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	N/A	Progeny from previously exposed parents were reared, so these F1 fish were not directly exposed.	
Metric 5:	Negative Control Response	N/A	F1 progeny from control F0 parents were represented. Progeny from previously exposed parents were reared so these F1 fish were not directly exposed.	
Metric 6:	Randomized Allocation	Medium	Random allocation of larvae was noted in section 2.5.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	The rearing of fish was presented in limited detail.	
Metric 8:	Consistency of Exposure Administration	High	It appears the treatment and control groups were handled the same.	
Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.	
Metric 10:	Exposure Duration and Frequency	High	These F1 larvae were not exposed to the chemical, but outcomes resulted from parents that had chronic exposures.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.	
Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	Source of these F1 fish were from previously exposed parents within the current experiment.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).	
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<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071151			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Values presented as mean Hatch rate, malformation rate, and survival were represented by three replicates of 100 embryos/larvae. Mean body weight is represented by triplicate groups with 20 larvae per replicate.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Housing from hatch to 7 dph was not well described.	
	Metric 17: Outcome Assessment Methodology	Low	Body weight of F1 larvae from the highest treatment concentration (F0 parents) was significantly lower than the control (Table 3). Methods for acquiring larval weight were not described. Criteria for malformation rate were not described.	
	Metric 18: Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information in the study to suggest animal attrition or health impacted results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.	
	Metric 22: Reporting of Data	High	Mean and SEM for output parameters are reported in table 3 on page 3/7.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	None			
Overall Quality Determination		High		

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

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<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071151			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 4).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Housing was described for the volume of containers. but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.	
	Metric 17: Outcome Assessment Methodology	High	Mortality was reported in Table 3.	
	Metric 18: Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and the control.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.	
	Metric 22: Reporting of Data	High	Mean HSI and standard error of the mean (table 2) were presented.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	This form is for mortality with results presented in Table 3 and in section 3.2 in the text.			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071151			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The nomenclature was reported correctly; CAS and chemical structure were not reported.	
Metric 2:	Test Substance Source	High	The source was reported from AccuStandard (Section 2.1), and the study verified the substance for nominal vs actual exposure concentrations (section 2.3).	
Metric 3:	Test Substance Purity	High	Purity from AccuStandard (New Haven CT) was reported as 99.6%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Solvent controls were used at a DMSO concentration of 0.005%.	
Metric 5:	Negative Control Response	High	Control metrics for growth (length, weight, and condition factor) are reported in Table 2 (page 3/7).	
Metric 6:	Randomized Allocation	Medium	The random allocation of larvae in treatment groups at the beginning of exposure was listed in section 2.2.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	The experimental systems were detailed in 2.2. The fish were moved to different units as they grew from hatch to 6 months post hatch for the 4 week spawning experiment.	
Metric 8:	Consistency of Exposure Administration	High	Exposure groups originate from the same cohort of spawned larvae. It appears the treatment and control groups were handled the same.	
Metric 9:	Measurement of Test Substance Concentration	High	Nominal concentrations were tested at 1, 3, 5, and 7 months to test for the chemical compound using GC/MS. The manuscript uses this data to report the actual exposure concentrations.	
Metric 10:	Exposure Duration and Frequency	High	Exposures cover from newly hatched larvae to sexual maturity with 4 weeks of spawning at 6 months post hatch.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	This long term study contained 3 treatment concentrations chosen to represent published field concentrations for DEPH.	
Metric 12:	Testing at or Below Solubility Limit	High	The highest nominal concentration was 100 ug/L, and the solubility listed in the scope is reported as 270 ug/L.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The source was not listed.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	The pre-treatment and treatment conditions appear to be the same for the newly hatched larvae (section 2.2).	
Metric 15:	Number of Organisms and Replicates per Group	Medium	There are three replicates with 6 fish per replicate (Table 2).	
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<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3071151		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	Housing was described for the volume of containers, but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.
Metric 17:	Outcome Assessment Methodology	Low	Length, weight, and condition factor of the F0 adults were not significantly different from the control after 7 months exposure to DEHP at concentrations of 4.2, 13.3, and 40.8 in females. There were inconsistent differences in male weight and condition factor at the middle treatment concentration when compared to the control (Table 2).
Metric 18:	Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted the results.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.
Metric 22:	Reporting of Data	High	Length, weight and condition factors for males and females are presented as means with standard error of the mean (table 2).
Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	This evaluation includes weight, length, GSI, and HSI		
<b>Overall Quality Determination</b>		<b>High</b>	

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

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Housing was described for the volume of containers but the authors did not report water quality parameters throughout the long-term exposure study, and they did not report the density (g/L) of the fish in each replicate.
	Metric 17: Outcome Assessment Methodology	High	Reproductive output was significantly lower at the two highest concentrations (figure 1) and GSI in females was significantly lower than control for the highest treatment concentration (table 2).
	Metric 18: Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information in the study to suggest animal attrition or health impacted results.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.
	Metric 22: Reporting of Data	High	Male and female GSI are presented as means with standard error of the mean (table 2) and egg output is also presented with SEM (figure 1).
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: None			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Guo, Y., Yang, Y., Gao, Y., Wang, X., Zhou, B. (2015). The impact of long term exposure to phthalic acid esters on reproduction in Chinese rare minnow ( <i>Gobiocypris rarus</i> ). Environmental Pollution 203(Elsevier):130-136.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Gobiocypris rarus</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Endocrine toxicity
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3071151

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Housing was described for the volume of containers, but the authors did not report water quality parameters throughout the long-term exposure study. They did not report the density (g/L) of the fish in each replicate.
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment for gene expression was well detailed and used a housekeeping gene to normalize background expression via the delta delta CT method. Organs for each gene primer were targeted (table 4).
	Metric 18: Consistency of Outcome Assessment	High	The assessment protocol appears to be assessed consistently among the treatment groups and control.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate that factors outside the chemical treatments impacted the results.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest animal attrition or health impacted results.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	ANOVA with Dunnetts post hoc were used to assess differences.
	Metric 22: Reporting of Data	High	Mean gene transcription and standard error of the mean (table 4) were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: This evaluation includes gene transcription and hormone concentrations.			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The environmental conditions were consistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The statistical methods were performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	None			
<b>Overall Quality Determination</b>		<b>High</b>		

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill ( <i>Lepomis macrochirus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316201			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance- Diisooctylphthalate (isomer of DEHP) was identified by chemical name and CASRN (27554-26-3).
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 Characterization				
	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 exposure. 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 experiment. Measured concentrations deviated from nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type- 96 hour acute toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response by the study authors and adequate to address the purpose of the study. For Diisooctylphthalate (isomer of DEHP) , a preliminary test was conducted, which indicated that it was not toxic below the water solubility limit. A corroborative test was then conducted exposing the bluegill to a single replicated concentration of Diisooctylphthalate (isomer of DEHP) representing its limit of water solubility.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
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<b>Study Citation:</b>	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill ( <i>Lepomis macrochirus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316201			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 blue gill in each test jar, and they were tested in duplicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable 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 on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	There was no mortality at the concentration tested. Therefore, statistical analysis was not conducted.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group. Negative findings were reported quantitatively (Table 4).
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Non-definitive LC 50 values reported based on a corroborative test (following negative findings from a preliminary test at concentration below the limit of water solubility) conducted by exposing bluegills to a single replicated concentration of Diisooctylphthalate (isomer of DEHP) representing its limit of water solubility.Diisooctylphthalate CASN 27554-26-3 is listed as a relevant form on the PECO within the Final scope for DEHP on page 99/148			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill ( <i>Lepomis macrochirus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316201			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by chemical name and CASRN.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure. Measured concentrations deviated from reported nominal concentrations.	
	Metric 8: Consistency of Exposure 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 experiment. 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 the spacing of exposure levels were justified for a dose response by study authors and adequate to address the purpose of the study. For DEHP, a preliminary test was conducted, which indicated that DEHP was not toxic below its water solubility limit. A corroborative test was then conducted exposing the bluegill to a single replicated concentration of DEHP representing its limit of water solubility.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 blue gill in each test jar, and they were tested in duplicates.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Bionomics,, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill ( <i>Lepomis macrochirus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316201			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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 on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	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 concentration below the limit of water solubility) conducted by exposing bluegills to a single replicated concentration of DEHP representing its limit of water solubility.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill ( <i>Lepomis macrochirus</i> ). Bulletin of Environmental Contamination and Toxicology 26(4):446-452.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	18064; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
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	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 group was not reported.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into 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. They also noted that "most of the LC50 values reported... do not reflect [t]he actual concentrations of the chemical which were in solution in the diluent", because "the acute toxicity of most of the chemicals tested was at concentrations above their water solubility".	
	Metric 8: Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured. They also noted that "most of the LC50 values reported... do not reflect [t]he actual concentrations of the chemical which were in solution in the diluent", because "the acute toxicity of most of the chemicals tested was at concentrations above their water solubility".	
	Metric 10: Exposure Duration and Frequency	High	Standard test durations were used (24h and 96h).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	No information is provided on the number of exposure groups and the spacing of exposure levels, but cited methods suggest using a minimum of 5 treatment levels.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The authors reported this as "precipitate". However, the highest reported LC50 is 2.1 mg/L, compared to the solubility reported in the DBP Final Scope of 11.2 mg/L at 25C.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor uncertainties about the source and characteristics of test organisms because the authors use a generalized description for all reported tests.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms for the 48 hours prior to testing.	

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<b>Study Citation:</b>	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill ( <i>Lepomis macrochirus</i> ). Bulletin of Environmental Contamination and Toxicology 26(4):446-452.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	18064; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
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 Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	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 / 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.	
Domain 7: Data Presentation and Analysis				
	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	The occurrence of unexpected outcomes was not addressed.	
Additional Comments:	None			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish ( <i>Lepomis macrochirus</i> ). :379-392.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	18050; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name and molecular weight.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Study authors reported using a concurrent negative control group, but acetone was used to dissolve the test chemical. Acetone was not reported as being used in the control.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported, although a background level of 20 cpm C-14 was detected prior to the exposure.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of the exposure was reported and appropriate for the study type (until a steady state was reached).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one sublethal concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were thirty fish used with no replicates.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish ( <i>Lepomis macrochirus</i> ). :379-392.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	18050; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	Medium	It wasn't clear how steady state was determined. For example, if there was subsampling or a duplicate exposure.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Fish were fed during the exposure. This factor may influence the results.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis is not typically performed for this type of endpoint.
	Metric 22: Reporting of Data	Medium	Only the overall BCF was reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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	High	The DEHP was high-purity (>99%) from a commercial source.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.	
Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.	
Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.	
Metric 8:	Consistency of Exposure Administration	High	Details of the exposure are provided and are consistent among study groups.	
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.	
Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations allowed for calculation of an LC50.	
Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of the concentrations was provided, and care was taken to ensure minimal degradation or loss of the test substance 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 Conditions	Low	Minimal details on pretreatment were provided.	
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5774391			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).
Domain 5: Outcome Assessment				
	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 Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.
	Metric 22:	Reporting of Data	Low	Results provided were minimal with authors noting specific concentrations and no acute toxicity.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The author provided discussion on different results measured in other papers.
Additional Comments:	DEHP was not acutely toxic to trout at the highest tested concentration (at/above water solubility). There were control, medium, and high test concentration treatments. Very limited data provided.			
Overall Quality Determination		High		

<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DEHP was identified by CASRN.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not reported if the DEHP was analytically verified.	
	Metric 3: Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7% The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study authors reported the use of an appropriate negative control in which no DEHP was used.	
	Metric 5: Negative Control Response	High	The negative control response of the preliminary study was reported in Table III and was adequate for the outcome of interest.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into test chambers for the preliminary study.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations. The test chambers were not described in detail, nor were the flow rates or other environmental conditions.	
	Metric 8: Consistency of Exposure Administration	Low	Test chambers were not adequately described in the preliminary study, nor were other test conditions. Analytical measurements were performed on days 6 and 15 of the study. The nominal concentration of the highest concentration was reported to be 40ug/L, and the measured concentrations on days 6 and 15 were 1.1 and 0.89ug/L respectively. This large difference creates concern about the consistency of the exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Gas chromatography techniques were used for analytical measurements in the highest test concentration only. Other test concentrations were not measured in the preliminary study. The test concentrations measured were vastly different from the nominal concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the preliminary study was reported to be 18 days. This was adequate to determine definitive test concentrations.	
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric	Rating	Comments	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the preliminary study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentration in the highest test level, which was the only level measurements were made. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.
	Metric 12:	Testing at or Below Solubility Limit	Low	Test concentrations were believed to be below the water solubility level. Testing was done after the preliminary test, and the water solubility limit for the dilution water was determined to be 23ug/L, which would place the highest test concentration above the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the preliminary study.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were two replicates for each test concentration and control with 10 embryos each, for a total of 20 embryos for each test concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The characteristics of the soft blended water used as the dilution water were reported in Table I. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. Feeding regimen, temperature, DO, and other water quality parameters were not discussed in the preliminary study.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest—egg and fry mortality.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for the preliminary study were limited. It was not reported when hatch was monitored or how often survival was checked for and determined.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated to test conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	11328250

Domain	Metric	Rating	Comments
	Metric 21: Statistical Methods	Low	It was not reported if statistical analysis was conducted for the preliminary study. Raw data is provided in Table III.
	Metric 22: Reporting of Data	High	Data for the exposure response and the control response of the preliminary study are provided in Table III and are adequate for the outcomes of interest.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not provide measures of variability for this portion of the study.

Additional Comments: This evaluation was for the preliminary early life-stage study on rainbow trout embryos. The preliminary study was conducted for 18 days and assessed percent hatch and survival of the fry. Little information was provided on when the organisms were monitored for hatch and survival. Nominal concentrations were drastically different from measured concentrations at the highest test level, which was the only test level monitored in the preliminary study.

## Overall Quality Determination

## Medium



<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric		Rating	Comments
Domain 1: Test Substance	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not reported if the DEHP was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7%. The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.
Domain 2: Test Design	Metric 4:	Negative Controls	Low	The study authors reported the use of a negative control. However, control samples had low level DEHP contamination.
	Metric 5:	Negative Control Response	High	The negative control response of the definitive study was reported in Table IX and was adequate for the outcome of interest. Control results were also reported in Figures 5 and 6.
	Metric 6:	Randomized Allocation	Low	It was reported the embryos were impartially allocated to the test chambers five at a time until all cups contained five eggs. This was continued until all chambers contained 50 eggs.
Domain 3: Exposure Characterization	Metric 7:	Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations, thus the low rating. Test chambers for embryos consisted of incubator cups suspended in the test chambers. Each replicate test chamber was 30.5cm x 15.7cm with a water depth of 15.7cm.
	Metric 8:	Consistency of Exposure Administration	Low	Test concentrations were reported to have 25-52% recovery from nominal concentrations. The two lowest test levels were only sampled on days 0, 1, and 7 due to inconsistencies in the measurements. The low and inconsistent recovery rates along with the detection of DEHP in the controls created concerns about consistency of the administration.
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric	Rating	Comments	
	Metric 9: Measurement of Test Substance Concentration	Low	Gas-liquid chromatography techniques were used for analytical measurements. Samples were taken on days 0, 1, and 7, and then approximately every 7 days after that, as well as at test termination. Sampling was discontinued for the two lowest test levels beginning on day 14, at request of the study sponsor.	
	Metric 10: Exposure Duration and Frequency	Low	The study duration was 70 days total or until 35 days post hatch. This was shorter than is the typical 60 days post hatch. The study was ended early at the request of the study sponsor due to inconsistent analytical measurements.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the preliminary study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentrations. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were reported to be below the water solubility limit of DEHP. Study authors tested solubility in the dilution water and determined it to be 23ug/L. The highest nominal concentration was reported to be 20ug/L, and the measured concentration was much lower than this.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the preliminary study.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were four replicates for each test concentration and eight replicates for the control. There were 50 embryos in each test chamber. After hatch, excess fry were discarded, and there were 25 fry per test chamber.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The characteristics of the soft blended water used as the dilution water were reported in Table I. Water quality measurements were reported in Table VI. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. The fish were fed starting on day 47 of the study. They were fed brine shrimp nauplii for the first six days and then Salmon Starter was fed in addition to the brine shrimp.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest—35d post hatch fry length and fry weight.	
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
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. At the termination of the study on day 35, fish were euthanized then blotted on paper towels to remove excess moisture. They were then weighed. Lengths were also determined at this point by measuring from the tip of the snout to the caudle peduncle.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated to test conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analysis" section and were appropriate for the outcomes of interest.
	Metric 22:	Reporting of Data	High	
	Metric 23:	Explanation of Unexpected Outcomes	High	Data for the exposure response and the control response of the definitive study are provided in Table IX and are adequate for the outcomes of interest. Data was also reported in Figures 5 and 6.
				Study authors did not report any unexpected outcomes. Variability was reported in Table IX.
Additional Comments:	This evaluation was for the definitive early life-stage study on rainbow trout embryos. The definitive study was conducted for 70 (35 days post hatch) days and mean wet weight and length were assessed. Nominal concentrations were drastically different from measured concentrations creating concern about consistency.			
Overall Quality Determination			Medium	

<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the DEHP was reported to be BASF Aktiengesellschaft. It was not reported if the DEHP was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of DEHP used for the diluter systems was reported to be 99.7% The purity of the DEHP used for the fortification samples and to prepare the analytical samples was reported to be 99.8%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The study authors reported the use of a negative control. However, it was reported that control samples had low level DEHP contamination.
	Metric 5:	Negative Control Response	High	The negative control response of the definitive study was reported in Table VIII and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was reported the embryos were impartially allocated to the test chambers five at a time until all cups contained five eggs. This was continued until all chambers contained 50 eggs.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	A 2L proportional diluter system was used to distribute the test substance at the proper concentrations for each test level. Diluter stock solutions were prepared using an experimental column saturator system. The stock solution was pumped directly to a chemical mixing box, and this became the highest concentration. It was reported that measured concentrations were significantly lower than the nominal concentrations, thus the low rating. Test chambers for embryos consisted of incubator cups suspended in the test chambers. Each replicate test chamber was 30.5cm x 15.7cm with a water depth of 15.7cm.
	Metric 8:	Consistency of Exposure Administration	Low	Test concentrations were reported to have 25-52% recovery from nominal concentrations. The two lowest test levels were only sampled on days 0, 1, and 7 due to inconsistencies in the measurements. The low and inconsistent recovery rates along with the detection of DEHP in the controls created concerns about consistency of the administration.
	Metric 9:	Measurement of Test Substance Concentration	Low	Gas-liquid chromatography techniques were used for analytical measurements. Samples were taken on days 0, 1, and 7, and then approximately every 7 days after that, as well as at test termination. Sampling was discontinued for the two lowest test levels beginning on day 14, at request of the study sponsor.
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain		Metric	Rating	Comments
	Metric 10:	Exposure Duration and Frequency	Low	The study duration was 70 days total or until 35 days post hatch. This was shorter than is the typical 60 days post hatch. The study was ended early at the request of the study sponsor due to inconsistent analytical measurements.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported there were five test concentrations in the definitive study. Results were presented in terms of the nominal concentrations. Analytical measurements were largely different from the nominal concentrations. Therefore, it is unknown what the spacing would be with the measured concentrations in the other levels.
	Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were reported to be below the water solubility limit of DEHP. Study authors tested solubility in the dilution water and determined it to be 23ug/L. The highest nominal concentration was reported to be 20ug/L, and the measured concentration was much lower than this.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Unfertilized eggs from three female trout, and sperm from five male trout were reported to be from Mt. Lassen Trout Farm in Red Bluff, CA, USA.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to exposure conditions prior to the start of the definitive study.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates for each test concentration and eight replicates for the control. There were 50 embryos in each test chamber. After hatch, excess fry were discarded, and there were 25 fry per test chamber.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The characteristics of the soft blended water used as the dilution water were reported in Table I. Water quality measurements were reported in Table VI. The embryos were kept in a dark environment until hatch when a photoperiod of 16L:8D was implemented. The fish were fed starting on day 47 of the study. They were fed brine shrimp nauplii for the first six days and then Salmon Starter was fed in addition to the brine shrimp.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest—egg and fry mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Total number of fry that hatched was determined on day 35 of the study. Fry mortality was monitored daily.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated to test conditions.
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<b>Study Citation:</b>	Cohle, P., Stratton, J. (1992). Early Life-Stage Toxicity of DEHP (CAS No. 117-81-7) to Rainbow Trout ( <i>Oncorhynchus mykiss</i> Walbaum 1792) in a Flow-Through System.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	11328250			
Domain	Metric		Rating	Comments
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Statistical Analysis" section and were appropriate for the outcomes of interest.
	Metric 22:	Reporting of Data	High	Data for the exposure response and the control response of the definitive study are provided in Table VIII and are adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study authors did not provide measures of variability for this portion of the study.
Additional Comments:	This evaluation was for the definitive early life-stage study on rainbow trout embryos. The definitive study was conducted for 70 (35 days post hatch) days and assessed percent hatch and survival of the fry. Nominal concentrations were drastically different from measured concentrations creating concern about consistency.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure are provided and are consistent among study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations allowed for evaluation of hatchability, survival, and weight.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentrations was provided, and care was taken to ensure minimal degradation or loss of test substance 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 Conditions	Low	Minimal details on pretreatment were provided.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5774391			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.	
	Metric 17: Outcome Assessment Methodology	High	The outcome of interest (survival) was appropriate.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups and environmental conditions are provided.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were appropriate.	
	Metric 22: Reporting of Data	High	Results for all treatments and outcomes were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes, and variance was reported. Authors provided discussion on different results measured in other papers.	
Additional Comments:	Trout embryos were exposed to DEHP for 90d with subsequent evaluation of hatchability, survival, and larval weight. No differences in survival were observed in trout exposed to the mean DEHP concentration. Survival ranged from 93-100%.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5: Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6: Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure are provided and are consistent among study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.
	Metric 10: Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The range of concentrations allowed for evaluation of hatchability, survival, and weight.
	Metric 12: Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of the concentrations was provided, and care was taken to ensure minimal degradation or loss of test substance 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 Conditions	Low	Minimal details on pretreatment were provided.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ; Embryo		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5774391		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.
	Metric 17: Outcome Assessment Methodology	High	The outcome of interest was appropriate (hatchability, survival, weight).
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were appropriate.
	Metric 22: Reporting of Data	High	Results for all treatments and outcomes were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes and, variance was reported. Author provided discussion on different results measured in other papers.
Additional Comments:	Trout embryos were exposed to DEHP for 90d with subsequent evaluations of hatchability and larval weight. No effects with DEHP exposure were observed. Hatchability ranged from 66-86%; Larval trout weight ranged from 599 mg in the high exposure tanks to 667 mg in the control tanks.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout ( <i>Salmo gairdneri</i> ) under flow-through conditions (final report) report no BW-83-3-1373.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ( <i>Salmo gairdneri</i> ); Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5530771			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The DEHP was identified by CASRN.
	Metric 2:	Test Substance Source	High	The DEHP was obtained from General Electric Company, Hudson Falls, NY on 11 and 18 December 1981.
	Metric 3:	Test Substance Purity	High	"100% active ingredient," was reported. Though absolute purity is doubtful, this can be interpreted as an indication of very high purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were included.
	Metric 5:	Negative Control Response	High	There was no unacceptable mortality in the controls.
	Metric 6:	Randomized Allocation	Medium	Trout were randomly distributed among aquaria.
Domain 3: Exposure Characterization				
	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 Administration	High	Exposures were administered consistently across substance groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured during experiment. Endpoints were based on measured concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The 96-h acute exposure was an acceptable duration.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations & the number of groups were acceptable to determine LC50 values.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The calculated LC50 (>0.32mg/L) was over the solubility limit for DEHP as reported in the Final Scope (0.28 mg/L).A dilution regimen for mixing the phthalates into solution was given in Appendix I. A combination of ultrasonication and mechanical mixing was used. Some of the high concentrations tested exhibited a visible film of undissolved phthalate. Although some measured concentrations were consistently lower than nominal, the measured concentrations were used to calculate LC50s.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Fish were obtained from commercial suppliers in Maryland & Montana. Lot numbers were given.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatization was for a minimum of 14 days in holding tanks.

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<b>Study Citation:</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout ( <i>Salmo gairdneri</i> ) under flow-through conditions (final report) report no BW-83-3-1373.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oncorhynchus mykiss</i> ( <i>Salmo gairdneri</i> ); Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5530771

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates per concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The test conditions adequate for the husbandry of rainbow trout.
	Metric 17: Outcome Assessment Methodology	High	The outcome was assessed appropriately (mortality).
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed consistently among study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	LC50 values were calculated by a customized computer program using moving average angle analysis, probit analysis, or binomial probability. Details of the program are not reported.
	Metric 22: Reporting of Data	High	Data was reported for all groups.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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	High	DEHP was high-purity (>99%) from commercial source.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure are provided and are consistent among study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	The exposure concentrations were measured using appropriate methods.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations allowed for calculation of an LC50.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentrations was provided, and care was taken to ensure minimal degradation or loss of the test substance 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 Conditions	Low	Minimal details on pretreatment were provided.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5774391			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).	
Domain 5: Outcome Assessment				
	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 Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were appropriate.	
	Metric 22: Reporting of Data	High	Results for all treatments and outcomes were reported.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Author provided discussion on different results measured in other papers.	
Additional Comments:	DEHP was not acutely toxic to medaka at the highest tested concentration (above water solubility). The authors assessed the control, medium, and high test concentration treatments. LC50 values were calculated but minimal raw data was provided.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1303977			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The DEHP was reported as 99% pure.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	There was no mention of control use in the methods, however, a control comparison was displayed in the results.	
	Metric 5: Negative Control Response	Medium	The biological response of the negative control groups was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	Few details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were standard.	
	Metric 12: Testing at or Below Solubility Limit	High	The exposure concentrations were at or 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 Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of replicates was 10 fish per treatment.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.	

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<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1303977			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted - reports for the acute exposure/vitellogenin assay are mainly qualitative.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: This evaluation was for serum proteins in adult fish.				

**Overall Quality Determination****Uninformative**



<b>Study Citation:</b>	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka ( <i>Oryzias latipes</i> ). Doctoral Dissertation:137.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5489073; Linked HERO ID(s): 5489073, 680110			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Nomenclature was reported in section 2.1. CAS number was not reported.
	Metric 2:	Test Substance Source	Low	The source was reported as from Sigma Aldrich, but it was not analytically verified by the lab.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The authors reported performing control and solvent controls.
	Metric 5:	Negative Control Response	Low	No control responses were reported for the DEHP exposures.
	Metric 6:	Randomized Allocation	Low	The allocation method was not random.
Domain 3: Exposure Characterization				
	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 Administration	High	Exposures were the same across treatments and control groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The methods report that concentrations of DEHP were verified, but no concentrations are reported for the DEHP exposures.
	Metric 10:	Exposure Duration and Frequency	High	The exposure (10 days) was reported to cover the normal period of embryo development for this species. Modal hatch was recorded between 11-14 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The authors did not report any concentrations from the DEHP exposures.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source was listed as from Carolina Biological Supply. These fish were bred to produce embryos used in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Embryo media and test water conditions were reported on page 45/158.
	Metric 15:	Number of Organisms and Replicates per Group	Low	No animal numbers were reported for the acute toxicity bioassays, but the methods indicated they were performed in triplicate.
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka ( <i>Oryzias latipes</i> ). Doctoral Dissertation:137.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5489073; Linked HERO ID(s): 5489073, 680110			
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	Methods for histology analysis were given in detail, but data on concentration specific incidence following exposure to DEHP were not provided.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for the DEHP exposure were not reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	No data were presented to indicate any outcomes from confounding variables.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	Authors were unable to perform statistics on these data. Negative findings were described in the text. "Acute Toxicity effects of up to 195.5 mg/L DEHP .... were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."	
	Metric 22: Reporting of Data	Low	There is no data presented on incidence of lesions following DEHP exposure.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Not enough data are presented for lesions at specific concentrations to determine if there were unexpected outcomes.	
Additional Comments:	The number of exposure groups and concentrations of DEHP tested were not provided. "Acute Toxicity effects of up to 195.5 mg/L DEHP .... were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."			
Overall Quality Determination		Uninformative		

<b>Study Citation:</b>	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka ( <i>Oryzias latipes</i> ). Doctoral Dissertation:137.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5489073; Linked HERO ID(s): 5489073, 680110			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was reported in section 2.1. CAS number was not reported.	
	Metric 2: Test Substance Source	Low	The source was reported as from Sigma Aldrich, but it was not analytically verified by the lab.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The authors reported performing control and solvent controls.	
	Metric 5: Negative Control Response	Low	No control responses were reported for DEHP exposures.	
	Metric 6: Randomized Allocation	Low	The allocation was not random.	
Domain 3: Exposure Characterization				
	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 Administration	High	The exposures were the same across treatments and control groups.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The methods report that concentrations of DEHP were verified, but no concentrations are reported for DEHP exposures.	
	Metric 10: Exposure Duration and Frequency	High	The exposure (10 days) was reported to cover the normal period of embryo development for this species. Modal hatch was recorded between 11-14 days.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The authors did not report any concentrations from the DEHP exposures.	
	Metric 12: Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was listed as from Carolina Biological Supply. These fish were bred to produce embryos used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Embryo media and test water conditions were reported on page 45/158.	
	Metric 15: Number of Organisms and Replicates per Group	Low	No animal numbers were reported for the acute toxicity bioassays, but the methods indicated they were performed in triplicate.	
Domain 5: Outcome Assessment				
	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.	

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<b>Study Citation:</b>	Patyna, P. J. (1999). Reproductive effects of phthalate esters in Japanese medaka ( <i>Oryzias latipes</i> ). Doctoral Dissertation:137.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5489073; Linked HERO ID(s): 5489073, 680110			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment for DEHP exposure was not reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	No data were presented to indicate any outcomes from confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Authors were unable to perform statistics on these data. Negative findings were described in the text. "Acute Toxicity effects of up to 195.5 mg/L DEHP .... were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."
	Metric 22:	Reporting of Data	Low	There is not enough data on embryo mortality to relate to a dose-response gradient.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Not enough data are presented for mortality at specific concentrations to determine if there were unexpected outcomes.
Additional Comments:	The number of exposure groups and concentrations of DEHP tested were not provided. "Acute Toxicity effects of up to 195.5 mg/L DEHP .... were also examined in the Japanese medaka, and caused no observable lesions or death at any of the tested concentrations."			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.	
	Metric 2: Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manufacturer in Japan).	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 2).	
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Authors provided limited details of the experimental systems and test media preparation. They described that DEHP was dissolved in ethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control solution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were acceptable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 weeks for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors only reported using 20 fish, but the use of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	The reporting of environmental conditions was insufficient to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology to document growth (body weight) and development (sex ratio and GSI) was not described in detail. For example, the authors did not describe/report how GSI is calculated or if the weight was wet weight. However, these omissions unlikely affected the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no evidence of differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes and S.E. was reported.
Additional Comments:	The overall goal of this study was to examine later-life health outcomes resulting from early-life exposure. The study examined the toxic effects of DEHP in adult Japanese medaka following a 3 week exposure during the larval (fry) stage. At their adult stage, mortality, sex ratio, and gonadosomatic index (GSI) were determined. This form evaluates growth and development: body weight, sex ratio, and GSI data.			

**Overall Quality Determination****Medium**

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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1333890

Domain	Metric	Rating	Comments
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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.	
	Metric 2: Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manufacturer in Japan).	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 1).	
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate (8%).	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Authors provided limited details of the experimental systems and the test media preparation. They described that DEHP was dissolved in ethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control solution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify the concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors did not offer a rationale for the selected exposure groups. The number of exposure groups was adequate (0.01, 0.1, 1, and 10 ug/ml), but based on the mortality results (20% for 0.1, 1, and 10 ug/ml), the spacing of exposure levels might not have been most appropriate for a dose response assessment.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit (DEHP water solubility = 0.27 mg/L).	
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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 weeks for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors only reported using 20 fish but the use of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	The reporting of environmental conditions was insufficient to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology to document mortality was not clearly described. The authors reported that mortality was measured at six months, but it is not clear if this was cumulative mortality or mortality on one single day or throughout the sixth month period.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There was no evidence of differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measure of variability for mortality.
Additional Comments:	The overall goal of this study was to examine later-life health outcomes resulting from early-life exposure. The study examined the toxic effects of DEHP in adult Japanese medaka following a 3 week exposure during the larval (fry) stage. At their adult stage, mortality, sex ratio, and gonadosomatic index (GSI) were determined. This form evaluates the mortality data.			

**Overall Quality Determination****Medium**

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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1333890

Domain	Metric	Rating	Comments
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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Authors only reported the chemical name. There was no other supporting information on chemical structure or CASRN.	
	Metric 2: Test Substance Source	High	The source of DEHP was identified as KANTO Chemical Co (which is a reagent manufacturer in Japan).	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	The authors did not describe the use of controls or the preparation of the control stock solution in the methods (section 2.2), but they reported control data in the results (text, Table 1, and Figure 2).	
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Authors provided limited details of the experimental systems and test media preparation. They described that DEHP was dissolved in ethanol (1 mg/mL) as a stock solution, but the concentration of the stock solution was not offered. Therefore, it is not possible to determine the final ethanol concentration in the diluted exposure solutions (nominal concentration of 0.01, 0.1, 1, and 10 ug/mL). They did not describe how the control solution was prepared or the content of ethanol in the control group. In addition, only half of the test solution was exchanged three times a week. Finally, they did not analytically verify concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Authors did not describe the exposure administration in detail across treatment groups including the control group.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate (3 weeks during the larval/fry stage) for the study type (evaluation of toxic effects in the adult stage following exposure in the fry stage).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were acceptable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
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<b>Study Citation:</b>	Chikae, M., Ikeda, R., Hatano, Y., Hasan, Q., Morita, Y., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate, $\gamma$ -hexachlorocyclohexane, and 17 $\beta$ -estradiol on the fry stage of medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 18(1):9-12.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333890			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Low	The source of the adult pair that was used to generate the embryos & fry used in this study was a pet shop. In addition, the authors only used a single pair of adults to generate the embryos & fry.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The authors acclimatized the adult pair used to generate the embryos and fry for 2 weeks for signs of illness and maturity. However, the authors did not indicate if/how after hatching fry fish were acclimatized before starting the exposure.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors only reported using 20 fish, but the use of replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was insufficient to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology to document growth (body weight) and development (sex ratio and GSI) was not described in detail. For example, the authors did not describe/report how GSI is calculated or if the weight was wet weight. However, these omissions unlikely affected the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There was no evidence of differences among the study groups in environmental conditions.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes and S.E. was reported.	
Additional Comments:	The overall goal of this study was to examine later-life health outcomes resulting from early-life exposure. The study examined the toxic effects of DEHP in adult Japanese medaka following a 3 week exposure during the larval (fry) stage. At their adult stage, mortality, sex ratio, and gonadosomatic index (GSI) were determined. This form evaluates growth and development: body weight, sex ratio, and GSI data. This form is for the sex ratio outcome.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Shioda, T., Wakabayashi, M. (2000). Evaluation of reproductivity of medaka ( <i>Oryzias latipes</i> ) exposed to chemicals using a 2-week reproduction test. Water Science and Technology 42(7-8):53-60.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1337871			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Uninformative	The control hatch/unhatch rate presented in Figure 1e indicates that 50% or more of the control eggs did not hatch. This is very unacceptable for a comparison of treatment effects.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The study provided limited details on the measures taken to appropriately prepare test concentrations.
	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 the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Uninformative	The number of exposure groups and the spacing of exposure levels were not conducive to the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolution.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Shioda, T., Wakabayashi, M. (2000). Evaluation of reproductivity of medaka ( <i>Oryzias latipes</i> ) exposed to chemicals using a 2-week reproduction test. Water Science and Technology 42(7-8):53-60.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1337871			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome 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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			
Overall Quality Determination		Uninformative		

<b>Study Citation:</b>	Shioda, T., Wakabayashi, M. (2000). Effect of certain chemicals on the reproduction of medaka ( <i>Oryzias latipes</i> ). Chemosphere 40(3):239-243.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683795			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The DEHP was sourced from GL Sciences.	
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Medium	The biological responses were adequate, but they varied among replicates – as did those in the treatment groups.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare the test concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Few details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	The exposure concentrations were not measured or the measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	Medium	Minor limitations in the exposure duration were identified. The exposure was not long enough to determine a dose response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and the spacing of exposure levels were justified, but no dose response was reported.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number used in studies of the same type, almost unacceptable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes.	
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<b>Study Citation:</b>	Shioda, T., Wakabayashi, M. (2000). Effect of certain chemicals on the reproduction of medaka ( <i>Oryzias latipes</i> ). Chemosphere 40(3):239-243.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683795			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were 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.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed, and the level of significance was 0.01.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: None				

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728529			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).	
	Metric 2: Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.	
	Metric 3: Test Substance Purity	High	The study reported a test substance purity of >99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.	
	Metric 5: Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 2-5.	
	Metric 6: Randomized Allocation	Low	Randomization was indicated in the study report, but the method was not indicated.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the test methods were described in adequate detail for this semi-static test (daily renewal).	
	Metric 8: Consistency of Exposure Administration	High	Exposures treatments were administered consistently across the control and test groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Mean-measured concentrations were provided in a supplemental Appendix A file, and based on test substance physical-chemical properties, measured concentrations are expected to be similar to nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle control) were adequate to show results relevant to the outcome of interest.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms is unclear, but there were at least 4 replicates of 4 test organisms per test concentration; and 4 larvae per test concentration were pooled for the analysis.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728529			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was consistent across treatment groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.	
	Metric 22: Reporting of Data	High	Data for all outcomes of interest and controls were presented.	
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.	
Additional Comments:	This evaluation is specific to the mechanistic endpoints examined in this experiment: gene expression (antioxidants and AChE biomarker).			
Overall Quality Determination		High		

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

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<b>Study Citation:</b>	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	4728529

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.
	Metric 22: Reporting of Data	High	Data for all outcomes of interest and controls were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.

Additional Comments: This form was for the mortality outcome reported in section 3.1.

**Overall Quality Determination**

**High**

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<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728529			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).	
	Metric 2: Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.	
	Metric 3: Test Substance Purity	High	The study reported a test substance purity of >99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.	
	Metric 5: Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 1 for growth and development.	
	Metric 6: Randomized Allocation	Low	Randomization was indicated in the study report, but the method was not indicated.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and test methods were described in adequate detail for this semi-static test (daily renewal).	
	Metric 8: Consistency of Exposure Administration	High	Exposures treatments were administered consistently across the control and test groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Measured analytical concentrations were provided in a supplemental Appendix and were slightly higher than nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle control) were adequate to show results relevant to the outcome of interest.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms is unclear, but there were at least 4 replicates of 4 test organisms per test concentration.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was consistent across treatment groups.	

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<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	4728529

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Data were expressed as means with standard deviations and analyzed with a one-way ANOVA; with post-hoc Fishers least significant difference test.
	Metric 22: Reporting of Data	High	Data for all outcomes of interest and controls were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Yang, W. K., Chiang, L. F., Tan, S. W., Chen, P. J. (2018). Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Science of the Total Environment 640-641:512-522.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728529			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl)phthalate (DEHP).	
	Metric 2: Test Substance Source	High	The test substance was obtained from Sigma-Aldrich, St Louis, MO.	
	Metric 3: Test Substance Purity	High	The study reported a test substance purity of >99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study reported using a negative vehicle control with 0.1% (v/v) acetone.	
	Metric 5: Negative Control Response	High	Negative control responses to the exposure were recorded in Fig 6.	
	Metric 6: Randomized Allocation	Low	Randomization was indicated in the study report for the exposure groups, but the method was not indicated.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and test methods were described in adequate detail for this semi-static test (daily renewal). The larvae were moved to 24-well plates for the behavioral test, and the test procedure was adequately described.	
	Metric 8: Consistency of Exposure Administration	High	Exposures treatments were administered consistently across the control and test groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Analytical concentrations were measured and reported in the Supplemental Material.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure, 21 days with daily renewals, was appropriate for the study outcomes.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing (20, 100 and 200 ug/L and vehicle control) were adequate to show results relevant to the outcome of interest.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organism larvae were obtained from laboratory-bred fish.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Free swimming larvae were acclimated for 24-hours, and pre-treatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of exposure test organisms is unclear, but there were at least 4 replicates of 4 test organisms per test concentration. There were 32 fish per test concentration for the locomotor activity behavior test.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were adequate for the study.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.	

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<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	4728529

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was consistent across treatment groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no reported outcomes unrelated to exposures that could impact the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Data for the locomotor activity were analyzed with nonparametric Kruskal-Wallis test.
	Metric 22: Reporting of Data	High	Data for all outcomes of interest and controls were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed unexpected outcomes in the paper.
Additional Comments:	None		

**Overall Quality Determination****High**



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

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<b>Study Citation:</b>	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 16(3):141-145.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334110			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	Numbers were adequate although inconsistent; replicates were not reported. "Spawning eggs were collected within 1–3 h after the lights were turned on. Under these conditions, approximately 40 eggs per day were pooled from ten pairs, and all eggs pooled in a day were assigned to one treatment."
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	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 limited.
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	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.
<b>Additional Comments:</b>	Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help.This form is for hatch success.			
<b>Overall Quality Determination</b>			<b>Medium</b>	

<b>Study Citation:</b>	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 16(3):141-145.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334110			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	The study provided few details on the exposure administration, notably, how long the embryos were exposed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	It was not clear if the post hatch solution contained DEHP.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit with the use of ethanol.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There were minor reservations over using pet store fish to supply test embryos.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Eggs were pooled on different days for each treatment, which seems quite inconsistent for a pretreatment.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers were adequate although inconsistent; replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
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<b>Study Citation:</b>	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 16(3):141-145.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334110

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
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	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 16(3):141-145.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334110			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	The study provided few details on the exposure administration, notably, how long the embryos were exposed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	It was not clear if the post hatch solution contained DEHP.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit with the use of ethanol.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There were minor reservations over using pet store fish to supply test embryos.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Eggs were pooled on different days for each treatment, which seems quite inconsistent for a pretreatment.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers were adequate although inconsistent; replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
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<b>Study Citation:</b>	Chikae, M., Hatano, Y., Ikeda, R., Morita, Y., Hasan, Q., Tamiya, E. (2004). Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Pharmacology 16(3):141-145.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; orange-red variety; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334110

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
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	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments: Starting each treatment on a different day seems inconsistent. It was also not clear if the exposure was only to embryos. Yamamoto, 1967 may help.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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	High	DEHP was high-purity (>99%) from a commercial source.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with the control groups.
	Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure are provided and are consistent among study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.
	Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations and the duration of the study allowed for evaluation of survival as well as weight changes.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility though verification of concentrations was provided, and care was taken to ensure minimal degradation or loss of test substance 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 Conditions	Low	Minimal details on pretreatment were provided.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5774391			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.	
	Metric 17: Outcome Assessment Methodology	High	The outcome of interest was appropriate (survival, weight).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were appropriate.	
	Metric 22: Reporting of Data	High	Results for all treatments and outcomes were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	Authors provided discussion on different results measured in other papers.	
Additional Comments:	Medaka were exposed to DEHP over 168d followed by evaluation of survival and measurement of weights. Weights of DEHP-exposed medaka were lower than control fish weights.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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	High	The DEHP was high-purity (>99%) from a commercial source.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.
	Metric 5: Negative Control Response	High	There were no concerns or anomalies associated with control groups.
	Metric 6: Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure are provided and are consistent among study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.
	Metric 10: Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The range of concentrations allowed for calculation of survival probability.
	Metric 12: Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility though verification of the concentrations was provided, and care was taken to ensure minimal degradation or loss of the test substance 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 Conditions	Low	Minimal details on pretreatment were provided.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5774391		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Study conditions (physiochemical characteristics, feeding details) are well documented.
	Metric 17: Outcome Assessment Methodology	High	The outcome of interest (survival) was appropriate.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were appropriate.
	Metric 22: Reporting of Data	High	Results for all treatments and outcomes were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported no unexpected outcomes and reported variance. Authors provided discussion on different results measured in other papers.
Additional Comments:	Medaka were exposed to DEHP over 168 followed by assessment of survival across five sampling periods. Survival of fish exposed to DEHP was not reduced below control fish survival.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1303977			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The DEHP was reported as 99% pure.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	There was no mention of control use in the methods; however, a control comparison was displayed in results.	
	Metric 5: Negative Control Response	Medium	The biological response of the negative control groups was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	Few details of the exposure administration were reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were standard.	
	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	High	The test organisms were adequately described and were obtained from a reliable source.	
	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	30 fish per treatment were exposed, but only one tank (one technical replicate) was used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported on to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.	

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<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1303977			
Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted. All data are provided for treatment and control fish in Table 1; however, there are no technical replicates.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	There was no measure of variability reported for the mortality endpoint (Table 1) because all fish (30 at start) were housed in one tank so their was no technical replicate.

Additional Comments: This evaluation is for mortality of fish from the chronic exposure (reported in Table 1).

## Overall Quality Determination

## Uninformative

<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1303977			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The DEHP was reported as 99% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	There was no mention of control use in the methods, however, a control comparison was displayed in results.
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control groups was reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Low	Few details of the exposure administration were reported.
	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 the spacing of exposure levels were standard.
	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	High	The test organisms were adequately described and were obtained from a reliable source.
	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 replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported on to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.

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<b>Study Citation:</b>	Kim, E. J., Kim, J. W., Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka ( <i>Oryzias latipes</i> ) exposed to di-2-ethylhexyl phthalate. Environment International 28(5):359-365.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1303977		
Domain	Metric	Rating	Comments
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 exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments:	This evaluation is for serum proteins.		

**Overall Quality Determination****Uninformative**

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

Domain	Metric	Rating	Comments
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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed for GSI only.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group for GSI. Other results were in the narrative.
	Metric 23: Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and a measure of variability for GSI was reported in Table 2

Additional Comments: This evaluation is for the gonadal development, GSI.

## Overall Quality Determination

## Medium



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

Domain	Metric	Rating	Comments
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 exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was not performed to test for differences in mortality.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	Variability was reported in Table 1.

Additional Comments: This evaluation was for body weight.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Metcalf, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka ( <i>Oryzias latipes</i> ). <i>Environmental Toxicology and Chemistry</i> 20(2):297-308.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333925			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1: Test Substance Identity	High	The chemical was identified as "the phthalic acid, diethylhexyl phthalate (DEHP)."	
	Metric 2: Test Substance Source	High	The chemical was purchased from Sigma-Aldrich.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design	Metric 4: Negative Controls	High	A solvent (acetone) control was included in the study.	
	Metric 5: Negative Control Response	High	Control responses were shown in Table 5.	
	Metric 6: Randomized Allocation	Low	Authors did not state random allocation.	
Domain 3: Exposure Characterization	Metric 7: Experimental System/Test Media Preparation	High	Environmental conditions were adequately described in the primary reference and in cited methodology (Gray and Metcalfe 1997).	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were adequately explained and appeared to be consistent.	
	Metric 9: Measurement of Test Substance Concentration	Low	The DEHP concentration was not reported (authors attempted to measure but were unsuccessful due to background contamination).	
	Metric 10: Exposure Duration and Frequency	High	This was approximately a 90 day exposure (until fish reached 1.5 cm in length) with renewal of dosing solution every two days.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	Different DEHP doses are stated in Table 5 (0.5, 1, and 5 ug/L) vs DEHP doses stated in Tables 1 and 6 (500, 1000, 5000 ug/L). It is unclear if doses stated in Table 5 are in the wrong units.	
	Metric 12: Testing at or Below Solubility Limit	Low	It is unclear if the doses reported in Table 5 are in the correct units. This would affect whether DEHP exceeded solubility in water.	
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	Medium	The source of the stock were described in the cited paper (Gray and Metcalfe 1997). Authors did not report separating males from females or equalizing the number of starting males and females at initiation of experiment.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Authors report rearing eggs in ERM in petri dishes to hatch and then starting the exposure in a static renewal system 1 day post-hatch. Acclimation to the static renewal system was not stated.	
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<b>Study Citation:</b>	Metcalf, C. D., Metcalfe, T. L., Kiparissis, Y., Koenig, B. G., Khan, C., Hughes, R. J., Croley, T. R., March, R. E., Potter, T. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka ( <i>Oryzias latipes</i> ). Environmental Toxicology and Chemistry 20(2):297-308.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333925			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 60 fish per treatment with no tank replication.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Housing conditions were adequately stated in the primary paper and in the cited reference (Gray and Metcalfe 1997).	
	Metric 17: Outcome Assessment Methodology	Low	The methodology was not described for measurement of total length, wet weight, or calculation of condition factor.	
	Metric 18: Consistency of Outcome Assessment	High	Measurements were taken when fish reached 1.5 cm in length.	
Domain 6: Confounding / 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 (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Authors state significant difference (or lack thereof) in Table 5, but there was no explanation of what statistical test was conducted.	
	Metric 22: Reporting of Data	High	Total length and wet weight are shown in Table 5 for DEHP treatments and controls.	
	Metric 23: Explanation of Unexpected Outcomes	High	The averages and associated standard deviation shown in Table 5 seem reasonable for DEHP treatments and control treatment.	
Additional Comments:	None			
Overall Quality Determination		Low		

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

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Housing conditions were adequately stated in the primary paper and in a cited reference (Gray and Metcalfe 1997).
	Metric 17: Outcome Assessment Methodology	High	Methodology for determining the presence of ova-testis was described.
	Metric 18: Consistency of Outcome Assessment	High	Measurements were taken when fish reached 1.5 cm in length.
Domain 6: Confounding / 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	Low	The numbers of fish in 1 mg/L and 0.5 mg/L DEHP treatments were almost half of those in the control and 5 mg/L DEHP treatments.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Authors stated significant difference (or lack thereof) in Table 6, but there was no explanation of what statistical test was conducted.
	Metric 22: Reporting of Data	High	The number of females and males (and number of males with TO) are shown in Table 6 for DEHP treatments and control.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; ChgH-EGFP; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2298079			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The CAS numbers and structures for BBP, DBP, DEHP, DIDP, and DINP were reported.	
	Metric 2: Test Substance Source	High	The sources were reported.	
	Metric 3: Test Substance Purity	Low	The purity/grade were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both a blank control and a solvent control were used in the acute bioassays.	
	Metric 5: Negative Control Response	High	Control responses (blank and solvent) are shown in Figure 3. Positive control responses are shown in Figures 3, 4, and 5.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Preparation of the test substances and dilution into the test medium were not well described.	
	Metric 8: Consistency of Exposure Administration	High	Exposures appear to have been administered consistently.	
	Metric 9: Measurement of Test Substance Concentration	Low	Concentrations are reported as nominal.	
	Metric 10: Exposure Duration and Frequency	Low	Exposures were 24-hr for embryos, shorter than typical 72-96 hr 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 DPB) and slightly exceeded solubility (DIDP, DINP, DEHP). Methanol was utilized to increase solubility.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The source for the transgenic medaka was cited to Chen et al 2007 and Cheng and Chen 2013, but it was not well described in the cited sources.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimation of embryos in 24-well plates was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Each concentration was tested in triplicate with eight embryos per replicate.	
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; ChgH-EGFP; Larvae
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2298079

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The 24 well plates were described, but no other details on environmental conditions were provided.
	Metric 17: Outcome Assessment Methodology	Medium	Measurement of GFP in the liver of embryos was described in the methods. Anesthesia of embryos prior to imaging was not described.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and control groups at 72 hr of exposure.
Domain 6: Confounding / 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 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 bioassay.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The authors utilized Student's t-tests (alpha = 0.05) to determine significant difference of phthalate/E2 cotreatment activity from E2 activity. Other data analysis was performed according to ISO 20281.
	Metric 22: Reporting of Data	Medium	Solvent control and positive control data were shown in Figure 3. Responses for the BBP treatment were shown in Figure 4, and co-treatment responses for DBP, DEHP, DIDP, and DINP were shown in Figure 5.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Only one treatment concentration was reported (1.5 mg/L), but it was reported with mean +/- SEM.

Additional Comments: This form applies to BBP, DBP, DEHP, DIDP, and DINP.

**Overall Quality Determination**

**Medium**



<b>Study Citation:</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 202:79-84.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4742097			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	TThe test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No CASRN, structure, or other chemical descriptors were reported.	
	Metric 2: Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they measured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.	
	Metric 3: Test Substance Purity	Low	The test substance purity/grade was not reported in the study.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.	
	Metric 5: Negative Control Response	High	The researchers reported the negative control response adequately.	
	Metric 6: Randomized Allocation	Low	The study authors did not report randomized allocation of the study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.	
	Metric 8: Consistency of Exposure Administration	High	The test substance was administered consistently across the study groups.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using HPLC on a weekly basis.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration of 56 days was adequate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Uninformative	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control. Most importantly for the mortality outcome, the researchers did not test a range of concentrations to determine mortality.	
	Metric 12: Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.	
Domain 4: Test Organism				
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<b>Study Citation:</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 202:79-84.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4742097			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details were not provided, the fish for the study were selected based on uniformity of weight.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The fish were uniformly acclimated to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	The size of the test tank was not specified, so it is unclear whether it was adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.	
	Metric 17: Outcome Assessment Methodology	Uninformative	The researchers did not describe the mortality methodology. They did not describe a range-finding test. There was no rationale offered for the mortality assessment because examining mortality due to DEHP exposure was not a goal of the study.	
	Metric 18: Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for mortality.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups that could affect outcome assessment, except that the authors did not report control impurities (ambient levels of DEHP).	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The study results were presented as means with standard errors and analyzed with one-way ANOVA and Duncan's Multiple Range test as merited.	
	Metric 22: Reporting of Data	High	The data for all outcomes were presented.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these were adequately discussed by the study authors.	

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<b>Study Citation:</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 202:79-84.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	4742097		
Domain	Metric	Rating	Comments
Additional Comments:	This study investigated the effects of various levels of DEHP exposure on growth performance, blood parameters, antioxidant enzyme activities, immune responses, and resistance to the <i>Aeromonas hydrophila</i> challenge on yellow catfish <i>Pelteobagrus fulvidraco</i> . This form was used to evaluate the survival data reported in Table 2. Mortality assessment was not the goal of the study, methods were not described for the mortality assessment, there was not mention of a range-finding test that revealed mortality rate due to DEHP exposure; percent survival was only mentioned in the results.		

**Overall Quality Determination****Uninformative**

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

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

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

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

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

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

**Overall Quality Determination****High**

<b>Study Citation:</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). <i>Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology</i> 202:79-84.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	4742097		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test substance was only identified by name (Di-2-ethylhexyl phthalate, DEHP). No CASRN, structure, or other chemical descriptors were reported.
Metric 2:	Test Substance Source	Low	The researchers obtained the test substance from Bellefonte, PA, but while they measured the test concentrations (metric 9), they did not verify the raw chemical obtained from Bellefonte.
Metric 3:	Test Substance Purity	Low	The test substance purity/grade was not reported in the study.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The researchers used a solvent-treated group (tween 80) as a control.
Metric 5:	Negative Control Response	High	The researchers reported the negative control response adequately.
Metric 6:	Randomized Allocation	Low	The study authors did not report randomized allocation of the study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The study adequately described dissolving the test substance in tween 80 (0.1 mg/L), with semi-static renewal three times a week and weekly measurement of test concentrations via HPLC.
Metric 8:	Consistency of Exposure	High	The test substance was administered consistently across the study groups.
Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using HPLC on a weekly basis.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration of 56 days was appropriate for the study type and assessment of growth performance.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The researchers used a control and two exposure concentrations of 0.1 and 0.5 mg DEHP/L. The test concentrations were within range of ambient concentrations (0.1 to 4 mg/L of DEHP reported in surface waters). DEHP was dissolved in 0.1 mg/L tween (carrier). While the researchers reported adding tween 80 to the control (solvent control), they did not specify what concentration of tween was present in the control.
Metric 12:	Testing at or Below Solubility Limit	High	A solvent, tween 80, was used to dissolve DEHP, which was prepared in concentrations of 0.1 and 0.5 mg/L, because the water solubility limit of DEHP is 0.270 mg/L.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The test organisms were obtained from a fish farm in Jiaxing, China. While age and other details not provided, the fish for the study were selected based on uniformity of weight.
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<b>Study Citation:</b>	Yuan, L., Li, M., Meng, F., Gong, Y., Qian, Y., Shi, G., Wang, R. (2017). Growth, blood health, antioxidant status, immune response and resistance to <i>Aeromonas hydrophila</i> of juvenile yellow catfish exposed to di-2-ethylhexyl phthalate (DEHP). Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology 202:79-84.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pelteobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4742097			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The fish were uniformly acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates (3 replicates of 30) were sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The size of the test tank was not specified, so it is unclear whether it was adequate for biomass loading of 30 fish per tank, however, the controls were provided with the same conditions, so this is unlikely to have a substantial impact on results.
	Metric 17:	Outcome Assessment Methodology	High	The researchers looked at final body weight, weight gain, and specific growth rate of the test organisms to observe exposure effects on growth and development.
	Metric 18:	Consistency of Outcome Assessment	High	The study authors consistently assessed the test group and controls for growth and development.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups that could affect outcome assessment, except that the authors did not report control impurities (ambient levels of DEHP) or the source of water used in the exposures which could potentially affect some of the results.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study results were presented as means with standard errors and analyzed with one-way ANOVA and Duncan's Multiple Range test as merited.
	Metric 22:	Reporting of Data	High	The data for all outcomes were presented (section 3.1, Table 2).
	Metric 23:	Explanation of Unexpected Outcomes	Medium	There are minor uncertainties in the study results because it is not clear that controls were free from DEHP at ambient concentrations, thus skewing comparisons to the 0.1 mg/L test group. However, effects were observed in the 0.5 mg/L test group, and these were adequately discussed by the study authors.
<b>Additional Comments:</b>	This study investigated the effects of various levels of DEHP exposure on growth performance, blood parameters, antioxidant enzyme activities, immune responses, and resistance to the <i>Aeromonas hydrophila</i> challenge on yellow catfish <i>Pelteobagrus fulvidraco</i> . This form was used to evaluate the growth performance assessment (final body weight, weight gain, specific growth rate, feed efficiency ratio, and hepatosomatic index; Section 2.1 and Table 2).			

**Overall Quality Determination****High**

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

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.	
	Metric 2: Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.	
	Metric 3: Test Substance Purity	High	There was at least 95% purity.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was reported.	
	Metric 5: Negative Control Response	High	The control response was acceptable.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration was consistent across groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Sample extracts were analyzed by gas chromatography at the start and the end of the test.	
	Metric 10: Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.	
	Metric 12: Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.	
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	The environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature was reported without CASRN.
	Metric 2:	Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	The duration and the frequency of exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes were reported.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The environmental conditions were consistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis				
	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**

<b>Study Citation:</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316188			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified definitively by an accepted name [di(2- ethylhexyl) phthalate; DEHP].	
	Metric 2: Test Substance Source	Low	The study states "The fourteen phthalate esters, all clear colorless liquids, labeled 1A through 1N, 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 Manufacturers Association, Washington. D.C."However, specific details about the test substance source (manufacturer, batch/lot #, etc.,) were not reported, and test substance identities were not analytically verified.	
	Metric 3: Test Substance Purity	Low	Purities of the test substances were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Appropriate, concurrent negative control groups were used –"Duplicate control jars containing the same dilution water and maintained under the same conditions as the exposure jars, but containing 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 and dissolved oxygen concentrations remained comparable to the respective controls during exposures to solutions of phthalate esters 1E through 1N [the test substances."	
	Metric 6: Randomized Allocation	Medium	"Ten fathead minnows (population descriptions in Table 1) were randomly distributed to each test jar after the test solutions had been prepared."	
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 appropriate amount of test substance to achieve the desired concentration. Temperature was controlled. "Dilution water used was soft water reconstituted from deionized water" (characteristics given); test solutions were not aerated. Water temperature, pH, DO, hardness, alkalinity, specific conductance were all monitored. No measures were taken to prevent loss of test substance over the course of the exposure, and in fact the authors reported that "an appreciable loss of phthalate ester from solution occurred during each exposure. The concentrations of phthalate ester present in solution at the end of the exposures ranged from <4 to 68% of the 0-hour concentration."	
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<b>Study Citation:</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316188			
Domain	Metric	Rating	Comments	
	Metric 8: Consistency of Exposure Administration	Low	Since no measures were used to maintain a consistent exposure concentration over the course of the experiment, there is no way to know how confidently two different exposure groups can be compared. The 96hr concentration shows the minimum possible exposure the test organisms were subjected to, but since no intermediate measurements were reported, the rate at which the test substance was lost, and how consistently the loss occurred between groups is unknown.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Test substance concentrations were only measured at the beginning and end of the study (0-hr and 96-hr). Analytical methods were appropriate, and they were detailed in an appendix – "An aliquot of the concentrate was analyzed by gas-liquidchromatography with electron capture detection."	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration is in accordance with the cited methodology [Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).]	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	For "corroborative tests" (tests for chemicals which showed no acute toxicity at their limit of solubility), only one exposure concentration was tested. The test substances within the limit of solubility were tested in triplicate.	
	Metric 12: Testing at or Below Solubility Limit	High	No exposure concentrations were above the limit of solubility; as such, no solvents were necessary.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Complete details regarding test organism characteristics are lacking. Only the source ("fathead minnows were obtained from cultures maintained at EG&G, Bionomics, Wareham, Massachusetts") and the mean length and mean weight of the test organisms in each fish population lot were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All test organisms underwent the same pretreatment process, including a 14-day 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	Medium	10 fish were included in each treatment group, and for the corroborative tests, three replicates were conducted for each test substance.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate – well detailed on pages 2-4 of the study (16hr light /8hr dark photoperiod; dry food fed ad libitum during the pretreatment period; 22+/-1 °c temp; measured water parameters stated).	
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<b>Study Citation:</b>	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to fathead minnows.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316188			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Medium	The authors report the percentage mortality for each study group at 24hr, 48hr, 72hr and 96hr. Since repeated concentration measurements were not taken, the accuracy of a NOEC derived from this data is limited. A NOEC can be derived from the lowest reported concentration (96hr), but the actual exposure experienced by the test organisms could range anywhere between there and the highest reported (0hr) concentration –“Concentrations of phthalate esters measured in solution at the initiation of the exposures ranged from 52 to 91% of the nominal concentrations (IN and IF, respectively). An appreciable loss of phthalate ester from solution occurred during each exposure. The concentrations of phthalate ester present in solution at the end of the exposures ranged from <4 to 68% of the 0-hour concentration IE and IN, respectively).”
	Metric 18:	Consistency of Outcome Assessment	High	No significant effects occurred, though it seems that like experimental conditions, outcome assessment was carried out consistently across all study groups.”No significant effects were observed among fathead minnows exposed to a single concentration of phthalate esters IE through IN representative of each materials limit of water solubility”
Domain 6: Confounding / Variable Control				
	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 the analyses as indicated by the QA spiked sample analytical results. The pH values and dissolved oxygen concentrations remained comparable to the respective controls during exposures to solutions of phthalate esters IE through IN.”
	Metric 20:	Outcomes Unrelated to Exposure	High	No attrition of test organisms unrelated to the exposure was reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary (results presented were just percentage mortality, at 0%).
	Metric 22:	Reporting of Data	High	Data is reported for all study groups in the appendix.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes occurred.
Additional Comments:	None			
Overall Quality Determination			High	

<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows ( <i>Pimephales promelas</i> ) under flow-through conditions.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1316189		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance (Diisooctyl phthalate; isomer of DEHP) was identified only by nomenclature. No other information (CASRN, structure, etc.) was provided.
	Metric 2: Test Substance Source	Low	The test substance was received from the General Electric Company, Hudson Falls, New York, on 11 and 18 December 1981. The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	The test substance was reported as "100% active ingredient." It is doubtful that it is literally one hundred percent, but this can be taken to mean very pure as received from manufacturer.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls were used.
	Metric 5: Negative Control Response	High	There was no unacceptable mortality in controls.
	Metric 6: Randomized Allocation	Medium	"The test was initiated when ten fathead minnows were randomly distributed to each aquarium "
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The flow-through regime was described in detail. Stock solutions were prepared daily & were maintained throughout experiment.
	Metric 8: Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9: Administration Measurement of Test Substance Concentration	High	Test substance concentrations were measured pre-test and at 0h and 96h.
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure and/or the exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (96-h acute toxicity study for fathead minnows, extended to 144h according to study design).
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number and the spacing of exposure groups were adequate.
	Metric 12: Testing at or Below Solubility Limit	High	The highest dose chosen was at the solubility limit of the chemical as "communicated verbally to EG&G Bionomics from the Syracuse Research Corporation (1982)" – though some droplets of undissolved phthalate were seen at high concentrations. See Appendix I for details of the solubilizing apparatus used to disperse phthalates in exposure water.
Domain 4: Test Organism			
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<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows ( <i>Pimephales promelas</i> ) under flow-through conditions.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316189			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Fathead minnows were from in-house cultures. Length and wet weight measurements were given in Table 1.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The acclimation was performed in holding tanks for "a minimum of 14 days" for both control & exposed fish.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were described in detail & were adequate for the health of <i>P. promelas</i> .
	Metric 17:	Outcome Assessment Methodology	High	Fish were observed for mortality every 24 hours.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	No mortality was observed in any treatment group. Statistical analysis was not necessary.
	Metric 22:	Reporting of Data	High	Data for all outcomes was presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: This evaluation was for the non-definitive 96hr LC 50 value reported for DIOP.				

**Overall Quality Determination****High**

<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows ( <i>Pimephales promelas</i> ) under flow-through conditions.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1316189		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure, etc.) was provided.
Metric 2:	Test Substance Source	Low	The DEHP was received from the General Electric Company, Hudson Falls, New York, on 11 and 18 December 1981. The test substance identity was NOT analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	"100% active ingredient" was reported. It is doubtful that it is literally one hundred percent, but this can be taken to mean very pure as received from the manufacturer.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Negative controls were used.
Metric 5:	Negative Control Response	High	There was no unacceptable mortality in the controls.
Metric 6:	Randomized Allocation	Medium	"The test was initiated when ten fathead minnows were randomly distributed to each aquarium "
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The flow-through regime was described in detail. Stock solutions were prepared daily & were maintained throughout the experiment.
Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	High	Test substance concentrations were measured pre-test and at 0h and 96h.
Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure and/or the exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (96-h acute toxicity study for fathead minnows, extended to 144h according to study design).
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The number and the spacing of exposure groups were adequate.
Metric 12:	Testing at or Below Solubility Limit	High	The highest dose chosen was at the solubility limit of the chemical as "communicated verbally to EG&G Bionomics from the Syracuse Research Corporation (1982)" – though some droplets of undissolved phthalate were seen at high concentrations. See Appendix I for details of the solubilizing apparatus used to disperse phthalates in exposure water.
Domain 4: Test Organism			
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 control & exposed fish.
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<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of thirteen phthalate esters to fathead minnows ( <i>Pimephales promelas</i> ) under flow-through conditions.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316189			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 fish per group with 2 replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Test conditions were described in detail & adequate for the health of <i>P. promelas</i> .	
	Metric 17: Outcome Assessment Methodology	High	Fish were observed for mortality every 24 hours.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in attrition or other health outcomes.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	No mortality was observed in any treatment group. Statistical analysis was not necessary.	
	Metric 22: Reporting of Data	High	Data for all outcomes was presented.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	This evaluation is for the non-definitive 96hr LC 50 value reported for DEHP.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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	The test substance identity was not analytically verified.	
Metric 3:	Test Substance Purity	High	The DEHP was high-purity (>99%) from a commercial source.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Control groups (no chemical) were evaluated in concert with treatment groups. No solvents were used to necessitate a solvent control.	
Metric 5:	Negative Control Response	High	There were no concerns or anomalies associated with the control groups.	
Metric 6:	Randomized Allocation	Medium	Fish were randomly distributed among treatments and randomly subsampled for measurements.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	Sufficient detail is provided regarding the experimental design as well as the development of stock solutions. Water physiochemical characteristics were regularly measured and reported. DEHP concentrations were measured and reported.	
Metric 8:	Consistency of Exposure Administration	High	Details of the exposure are provided and are consistent among study groups.	
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate methods.	
Metric 10:	Exposure Duration and Frequency	High	The exposure was appropriate and followed standard ASTM protocols.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations allowed for calculation of an LC50.	
Metric 12:	Testing at or Below Solubility Limit	Medium	Some test concentrations were at or above solubility, though verification of concentrations was provided, and care was taken to ensure minimal degradation or loss of the test substance 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 Conditions	Low	Minimal details on pretreatment were provided.	
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5774391			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Test organisms follow standard ASTM protocol and are reported as fish loading (g/L).
Domain 5: Outcome Assessment				
	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 Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no variations or inconsistencies reported across study groups, and environmental conditions are provided.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were appropriate.
	Metric 22:	Reporting of Data	High	Results for all treatments and outcomes were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors provided discussion on different results measured in other papers.
Additional Comments:	DEHP was not acutely toxic to fathead minnows at the highest tested concentration (above water solubility). Control, medium, and high test concentration treatments were reported. The LC50 was calculated, but minimal raw data was provided.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071071			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity/grade were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).	
	Metric 5: Negative Control Response	Low	The authors did not report the actual survival rate of the control groups (water control or carrier/solvent control). The only information they offered about the embryo mortality was "In the first experiment, a 48-h exposure to one of three concentrations of phthalates did not result in any significant mortality compared to the controls (H = 6.16; p = 0.10)."	
	Metric 6: Randomized Allocation	Low	The embryo exposure was not detailed as random allocation.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Details in the preparation of the solutions for the embryonic DEHP experiment was lacking. But the authors did indicate that the exposure was carried out for 48 h at ambient temperature, and the chemicals were prepared fresh and were renewed at 24 h with a 90 %water change.	
	Metric 8: Consistency of Exposure Administration	High	Exposure administration appeared consistent among treatment and control groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were presented as nominal (1, 10, and 100 ug/L).	
	Metric 10: Exposure Duration and Frequency	High	This goal was to assess mortality in embryos (pre-hatch), therefore a 48-hour exposure was appropriate.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).	
	Metric 12: Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.	
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071071			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors reported that embryos were evenly distributed into 12 glass petri dishes per treatment group, and at 24 h, up to 3 embryos per replicate were collected. However, there is no clear indication of the total number of organisms (embryos) distributed to each of the 12 petri dishes.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology (counts of dead organisms) was not clearly described for the intended outcome of interest (mortality due to DEHP exposure). Briefly, the authors reported that the exposure was carried out for 48 h, and at 24 h, up to 3 individuals per replicate were collected, and at 48 h, the number of surviving embryos was assessed in each treatment. Unfortunately, there is not clear indication of whether dead organisms were counted at 24h or if that only happened at 48 h.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment and control groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influenced the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Authors reported that differences in survival and hatch were tested using a Mantel–Cox log-rank test.	
	Metric 22: Reporting of Data	Low	Embryo experiment: The mortality rate per treatment group was not reported. Results were not shown in a table or figure. In the text, the authors reported that a 48-h exposure to one of three concentrations of DEHP did not result in any significant mortality compared to the controls (H = 6.16; p = 0.10).	
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3071071

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the *dnmt* genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate embryo mortality.

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071071			
Domain	Metric		Rating	Comments
Domain 1: Test Substance	Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.
Domain 2: Test Design	Metric 4:	Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).
	Metric 5:	Negative Control Response	Medium	The authors reported the genomic methylation levels for the control group, which are believed to be adequate. However, the authors did not report the specific mRNA levels for the gene expression analysis.
	Metric 6:	Randomized Allocation	Low	The allocation method was not detailed as random allocation.
Domain 3: Exposure Characterization	Metric 7:	Experimental System/Test Media Preparation	Medium	Details in the preparation of the solutions for the embryonic DEHP experiment were lacking. But the authors did indicate that the exposure was carried out for 48 h at ambient temperature, and the chemicals were prepared fresh and renewed at 24 h with a 90 %water change.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatment and control groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were presented as nominal.
	Metric 10:	Exposure Duration and Frequency	High	The goal was to assess dmnt expression in embryos (pre-hatch), therefore a 48-hour exposure was appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).
	Metric 12:	Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.
Domain 4: Test Organism				
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071071			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors reported that embryos were evenly distributed into 12 glass petri dishes per treatment group, and that at 24 h, up to 3 embryos per replicate were collected. However, there is no clear indication of the total number of organisms (embryos) distributed to each of the 12 petri dishes.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (RNA extraction and real-time PCR) was clearly described for the intended outcome of interest (measuring expression of the dnmt genes to understand epigenetic mechanisms).	
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment and control groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influenced the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Authors used ANOVA and Tukey’s post-hoc for gene expression. Normality and homogeneity of variance were tested with Shapiro-Wilks and Levene’s test, respectively.	
	Metric 22: Reporting of Data	Low	Embryo experiment: The expression of dnmt per treatment group was not reported. Results were not shown in a table or figure. In the text, the authors reported that following a 24-h exposure, no differences were detected among groups for mRNA levels of dnmt1 (df = 3; H = 1.5; p = 0.68), dnmt3 (H = 3.9; p = 0.28), dnmt6 (H = 3.5; p = 0.33), dnmt7 (H = 3.5; p = 0.34), or dnmt8 (H = 0.51; p = 0.92).	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Embryo
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3071071

Domain	Metric	Rating	Comments
Additional Comments:	The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the dnmt genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate embryo expression of the dnmt genes. The authors did not assess global DNA methylation for the embryos.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3071071		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, chemical structure, or other chemical descriptors.
Metric 2:	Test Substance Source	Low	The source was not reported.
Metric 3:	Test Substance Purity	Low	The purity/grade were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).
Metric 5:	Negative Control Response	Medium	Cumulative mortality for the 14 day exposure in control groups was approximately 20%. This is not an acute test (10% requirement) and larval survival (80%) over this period is not unexpected.
Metric 6:	Randomized Allocation	Medium	The authors reported: "Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate."
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The authors offered details on using the stock solution to prepare the exposure solutions every 48 hours: the control water (ethanol added), the D1 water (DEHP dissolved in ethanol added to make a 1.0-ug/L solution), the D2 water (DEHP dissolved in ethanol added to make a 10.0-ug/L solution), and the D3 water (DEHP dissolved in ethanol to make a 100.0-ug/L solution).
Metric 8:	Consistency of Exposure Administration	High	Exposure administration appeared consistent among treatment and control groups.
Metric 9:	Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were presented as nominal.
Metric 10:	Exposure Duration and Frequency	High	Larval study: The study examined endpoints for mortality throughout 14 days in larvae [0 days post-hatch (dph)]. The authors explained that this life stage was chosen for the exposure because research conducted in zebrafish showed that there is high dnmt expression in the post-hatch stage (Smith et al. 2011), and investigating DNA methylation patterns was the main goal of the study.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The three concentrations were 1, 10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071071			
Domain	Metric	Rating	Comments	
	Metric 12: Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Laval experiment: The organisms were placed in 40 glass petri dishes arranged into 4 groups of 10. Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate. The 4 treatments were a carrier control, (n = 10), 1 lg/L DEHP (n = 10), 10 lg/L DEHP, (n = 10), and 100 lg/L DEHP (n = 10).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (counts of dead organisms) was clearly described and appropriate for the intended outcome of interest (mortality due to DEHP exposure).	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was reported with criteria for mortality and assessed the same across treatment and control groups. Briefly, the authors reported that each replicate was checked daily for mortalities (completely white body and/or lack of a heartbeat under a dissection microscope). Mortalities were recorded each morning before the water was changed, and the exposure was carried out for fourteen days post-hatch (*19–20 days total postfertilization).	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influenced the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	”Differences in survival and hatch were tested using aMantel–Cox log-rank test.”	
	Metric 22: Reporting of Data	High	Percent cumulative mortality is presented in Figure 2.	

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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3071071		
Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the <i>dnmt</i> genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate mortality in larvae.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3071071		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The authors named the compound as "di-2-(ethylhexyl) phthalate" on page 2/12 in the introduction. However, they did not report the CASRN, structure, or other chemical descriptors.
	Metric 2: Test Substance Source	Low	The source was not reported.
	Metric 3: Test Substance Purity	Low	The purity/grade were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Authors reported using both a water control and a carrier control (0.001 % ethanol solution).
	Metric 5: Negative Control Response	Medium	Control gene expression was not explicitly detailed, however, it is incorporated into the "normalized expression" of each dnmt gene as a fold expression value.
	Metric 6: Randomized Allocation	Medium	The authors reported: "Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate."
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The authors offered details on using the stock solution to prepare the exposure solutions every 48 hours: the control water (ethanol added), the D1 water (DEHP dissolved in ethanol added to make a 1.0-ug/L solution), the D2 water (DEHP dissolved in ethanol added to make a 10.0-ug/L solution), and the D3 water (DEHP dissolved in ethanol to make a 100.0-ug/L solution).
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatment and control groups.
	Metric 9: Measurement of Test Substance Concentration	Low	There was no measurement of treatment concentrations. All concentrations were presented as nominal.
	Metric 10: Exposure Duration and Frequency	High	Larval study: The study examined endpoints for mRNA expression of the dnmt genes and global DNA methylation throughout 14 days in larvae [0 days post-hatch (dph)]. The authors explained that this life stage was chosen for the exposure because research conducted in zebrafish showed that there is high dnmt expression in the post-hatch stage (Smith et al. 2011).
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The three concentrations were 1,10, and 100 ug/L. The authors indicated throughout the publication that these concentrations are all considered environmentally relevant as DEHP has been found in surface waters as high as 300 ug/L in Canadian freshwater systems (CCME 1999).
	Metric 12: Testing at or Below Solubility Limit	High	All bioassay concentrations were below the 0.27 mg/L solubility listed in the Final Scope. In addition, authors used a solvent (0.001% ethanol) to dissolve DEHP.
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	3071071		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Authors used animals from an in house breeding stock at the University of New Brunswick. Moreover, the authors also indicated that the organisms used, fathead minnows, are a model freshwater toxicological species ubiquitously found in North America with the large collection of knowledge about their biology in addition to being used extensively in toxicological research for over 50 years (Ankley and Villeneuve 2006).
Metric 14:	Acclimatization and Pretreatment Conditions	High	The methods described many critical aspects of housing and environmental variables, including: dissolved oxygen, light cycle, temperature, and pH.
Metric 15:	Number of Organisms and Replicates per Group	Medium	Laval experiment: The organisms were placed in 40 glass petri dishes arranged into 4 groups of 10. Four larval [0 days post-hatch (dph)] FHM were randomly placed in each petri plate. The 4 treatments were a carrier control, (n = 10), 1 lg/L DEHP (n = 10), 10 lg/L DEHP, (n = 10), and 100 lg/L DEHP (n = 10).
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	Animal care and environmental conditions were well described.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (RNA extraction, real-time PCR, DNA extraction, and global DNA methylation) was clearly described and adequate for the intended outcome of interest (measuring dnmt gene expression and DNA methylation to understand epigenetic mechanisms).
Metric 18:	Consistency of Outcome Assessment	High	The methods/techniques used were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was presented to indicate that environmental conditions or other factors influenced the outcome assessment.
Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented by the authors to indicate health outcomes or attrition unrelated to exposure influenced the results.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Authors used ANOVA and Tukey's post-hoc to analyze gene expression and DNA methylation data. Normality and homogeneity of variance were tested with Shapiro-Wilks and Levene's test, respectively.
Metric 22:	Reporting of Data	Low	The authors clearly reported the DNA methylation data in the text and Figure 2. However, they did not offer results of the dnmt gene expression data.
Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
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<b>Study Citation:</b>	Wood, R. K., Crowley, E., Martyniuk, C. J. (2015). Developmental profiles and expression of the DNA methyltransferase genes in the fathead minnow ( <i>Pimephales promelas</i> ) following exposure to di-2-ethylhexyl phthalate. <i>Fish Physiology and Biochemistry</i> 42(1):7-18.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Larvae
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3071071

Domain	Metric	Rating	Comments
Additional Comments:	The objectives of this study were to determine whether DEHP exposure affects survival and development via epigenetic mechanisms by measuring the expression of the dnmt genes and in vivo DNA methylation in fathead minnow embryos and larvae. This form was used to evaluate expression of the dnmt genes and global DNA methylation in larvae.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1014765			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified as di(2-ethylhexyl) phthalate (DEHP).	
	Metric 2: Test Substance Source	Low	The test substance source was Sigma-Aldrich, but the materials were not certified or analytically verified in the study.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative solvent control group.	
	Metric 5: Negative Control Response	Medium	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	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 the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was used.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Initial ages, lengths, and weights of the test organisms were not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for two days.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 test organisms per test concentration and no replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	

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<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Genotox (including DNA repair)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1014765

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 / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was performed and was appropriate for dataset.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group, and results were described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-ADME			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1014765			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified as di(2-ethylhexyl) phthalate (DEHP).	
	Metric 2: Test Substance Source	Low	The test substance source was Sigma-Aldrich, but the materials were not certified or analytically verified in the study.	
	Metric 3: Test Substance Purity	Low	The purity/grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative solvent control group.	
	Metric 5: Negative Control Response	Medium	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was used.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Initial ages, lengths, and weights of test organisms were not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for two days.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 test organisms per test concentration and no replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
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<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-ADME			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1014765			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed and was appropriate for the dataset.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group, and results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This evaluation is for the assessment of plasma testosterone and 17beta-estradiol following exposure to DEHP.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1014765			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name as di(2-ethylhexyl) phthalate (DEHP).	
	Metric 2: Test Substance Source	Low	The test substance was obtained from Sigma-Aldrich, but the chemical identity was not certified and not analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative solvent control group.	
	Metric 5: Negative Control Response	Medium	The biological response of the negative control group was adequate in that it was reported as not significantly different from any treatment result.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was used.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Age, length, and initial weights of test organisms were not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for two days.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 test organisms for each test concentration (control and the single test concentration). There were no replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	

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<b>Study Citation:</b>	Crago, J., Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow ( <i>Pimephales promelas</i> ) through a novel mechanism of action. <i>Aquatic Toxicology</i> 110-111:74-83.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1014765

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical methods were adequately described in section 2.5.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation is for GSI.

## Overall Quality Determination

## Medium

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

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<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	791717			
Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Low	Details of the outcome assessment protocol 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.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
	Metric 22:	Reporting of Data	Low	Data were only reported as a narrative.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	791717		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
Metric 22:	Reporting of Data	Low	Data were only reported as a narrative.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	791717			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was unclear if the author used a negative or a solvent control.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Researchers used a radio labelled chemical to monitor concentrations.	
	Metric 10: Exposure Duration and Frequency	High	A flow-through design with an appropriate duration were reported.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates (3) were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pimephales promelas</i> ; Adult
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	791717

Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted, which is typical for BCF assessments.
Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes, not for the controls.
Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Zanotelli, V., Neuhauss, S., Ehrenguber, M. (2010). Long-term exposure to bis(2-ethylhexyl)phthalate (DEHP) inhibits growth of guppy fish ( <i>Poecilia reticulata</i> ). Journal of Applied Toxicology 30(1):29-33.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Poecilia reticulata</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	697429			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The DEHP was procured from Sigma.	
	Metric 3: Test Substance Purity	High	Purity was reported as >98%, industrial grade.	
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 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	Medium	The experimental system and/or the test media preparation methods were adequately reported.	
	Metric 8: Consistency of Exposure Administration	Medium	It wasn't clear if control solutions were renewed with the same schedule as the chemical treatment solutions.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	Minor limitations in exposure frequency and duration of exposure were identified. Increased frequency of renewals may have been beneficial.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The high exposure concentration exceeded the water solubility limit.	
Domain 4: Test Organism				
	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 pretreatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were adequate.	

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<b>Study Citation:</b>	Zanotelli, V., Neuhauss, S., Ehrenguber, M. (2010). Long-term exposure to bis(2-ethylhexyl)phthalate (DEHP) inhibits growth of guppy fish ( <i>Poecilia reticulata</i> ). Journal of Applied Toxicology 30(1):29-33.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Poecilia reticulata</i> ; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	697429

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcomes of interest.
	Metric 18: Consistency of Outcome 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	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	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335887			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical grade was reported as analytical.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure 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 the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of organisms was adequate, but replicates were not used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
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<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335887			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test 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 Presentation and Analysis				
	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**

<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335887			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical grade was reported as analytical.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the exposure was reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The initial number of organisms was adequate, but replicates were not used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1335887		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335887			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical grade was reported as analytical.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	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 the spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were appropriate for evaluation of the specific outcomes of interest.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The initial number of organisms was adequate, but replicates were not used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	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, <i>Pseudobagrus fulvidraco</i> (Richardson). Journal of Applied Ichthyology 25(6):771-775.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water, Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Pseudobagrus fulvidraco</i> ; Adult		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1335887		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation is for AChE activity.			
<b>Overall Quality Determination</b>		<b>High</b>	

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

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

**Overall Quality Determination****Uninformative**

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

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were approximately 50 eggs per test chamber with 5 replicates per treatment and control.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	The test was conducted at 5C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest--time to embryo hatch, deformation rate, and growth.
	Metric 18: Consistency of Outcome Assessment	High	Hatch was monitored on days 22, 25, and 35 for the 5C studies, and tadpole development was assessed at the end of the study.
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 or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section.
	Metric 22: Reporting of Data	High	Exposure and control related findings were reported in Tables 10 and 11 and in Figure 6 and were adequate for the outcomes of interest.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study authors reported that some of the test substance started to leach into the test water and could potentially affect the outcomes.
Additional Comments:	This portion of the study was on the effect of DEHP on hatch time, tadpole deformities, and tadpole weights in embryos and the tadpoles that hatched from said embryos at 5C. The outcome selected was development/growth.		

**Overall Quality Determination****High**

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

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<b>Study Citation:</b>	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	7328184		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 10C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—egg and tadpole mortality.
Metric 18:	Consistency of Outcome Assessment	High	Hatch was monitored on days 9, 12, 16, and 21 for the 10C studies, and tadpole survival was assessed at the end of the study.
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 or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section.
Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 8 and 9 and were adequate for the outcomes of interest.
Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study authors reported that some of the test substance started to leach into the test water and could potentially affect the outcomes.
Additional Comments:	This portion of the study was on the effect of DEHP on mortality in embryos and the tadpoles that hatched from said embryos at 10C. The outcome selected was mortality.		
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	7328184		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	The test was conducted at 10C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–time to embryo hatch, deformation rate, and growth.
	Metric 18: Consistency of Outcome Assessment	High	Hatch was monitored on days 9, 12, 16, and 21 for the 10C studies and tadpole development was assessed at the end of the study.
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 or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis were described in the "Statistical methods" section.
	Metric 22: Reporting of Data	High	Exposure and control related findings were reported in Tables 8 and 9 and in Figure 5 and were adequate for the outcomes of interest.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study author reported that some of the test substance started to leach into the test water and could potentially affect the outcomes.
Additional Comments:	This portion of the study was on the effect of DEHP on hatch time, tadpole deformities, and tadpole weights in embryos and the tadpoles that hatched from said embryos at 10C. The outcome selected was development/growth.		
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	IVL, (2001). Further investigations on the influence of sediment-associated phthalate esters (DEHP and DINP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	7328184		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	The test was conducted at 5C with a 12L:12D photoperiod in synthetic lake water. The water was reported to be aerated. Temperatures, D.O.s, and pH levels were reported. It was not reported if the tadpoles were fed after the they hatched.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—egg and tadpole mortality.
Metric 18:	Consistency of Outcome Assessment	High	Hatch was monitored on days 22, 25, and 35 for the 5C studies, and tadpole survival was assessed at the end of the study.
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical analyses were described in the "Statistical methods" section.
Metric 22:	Reporting of Data	High	Exposure and control related findings were reported in Tables 10 and 11 and were adequate for the outcomes of interest.
Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was reported in the tables. Study author reported that some of the test substance started to leach into the test water and could potentially affect the outcomes.
Additional Comments:	This portion of the study was on the effect of DEHP on mortality in embryos and the tadpoles that hatched from said embryos at 5C. The outcome selected was mortality.		
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Temperature variations were evident.	
	Metric 20: Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tadpole.	
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.	
Additional Comments:	This evaluation was for survival.			
Overall Quality Determination		Low		

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

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<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	7978546		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	Temperature variations were evident.
Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	No statistics were conducted for motility.
Metric 22:	Reporting of Data	Uninformative	No quantitative data was presented on motility. No dose-specific qualitative data was presented either.
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was reported and reasonable, but there was some contamination in the control water.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not described adequately.	
	Metric 8: Consistency of Exposure Administration	Medium	The study provided few details on exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Water concentrations were measured, but concentrations varied considerably.	
	Metric 10: Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were confusing.	
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<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo		
<b>Health Outcome:</b>	ADME (biotransformation)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	7978546		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	Temperature variations were evident.
Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	N/A	Statistical analysis is not typical for this outcome.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tadpole.
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>Low</b>	

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

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

<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not described.	
	Metric 8: Consistency of Exposure Administration	Medium	The study provided few details on exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Water concentrations were measured, but concentrations varied considerably.	
	Metric 10: Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	Medium	The method and the timing of determining hatch were not clear.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Temperature variations were evident.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Fungal infections may have skewed the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Sufficient data were provided to conduct an independent statistical analysis, but removal of some samples due to infection was somewhat arbitrary.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control tad-pole.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: This form was for survival and hatch.				
<b>Overall Quality Determination</b>			<b>Medium</b>	

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

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

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

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

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

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

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

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

<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
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Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
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	Metric 10: Exposure Duration and Frequency	Low	The duration was appropriate, but lack of renewals led to fungal infections.	
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Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The test organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	

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

<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
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	Metric 7: Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
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	Metric 13: Test Organism Characteristics	Low	The test organisms were wild caught. It was unclear at what development stage the exposure was initiated.	
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	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	

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

<b>Study Citation:</b>	IVL, (1997). The influence of sediment-associated phthalate esters (DEHP and DIDP) on hatching and survival of the moorfrog, <i>Rana arvalis</i> .			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	7978546			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	High	DEHP was analytically verified using GC-MS.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
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	Metric 7: Experimental System/Test Media Preparation	Medium	The methods for preparation of the test media were described in adequate detail, but the experimental system was not adequately described.	
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	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 organisms with 5 replicates for 50 total.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Inconsistencies in temperature were a concern.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	

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

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

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

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

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

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

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

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

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

<b>Study Citation:</b>	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5508563			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by the chemical name. No other information was provided.
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Kebo-Grave (Sweden), but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups were adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set up and test media preparation methods were reported but did not completely account for physical-chemical properties.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using capillary gas chromatography. Measured concentrations were similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 8 treatment groups and two controls (sediment plus ethanol and water only). The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	Medium	No effects on biological responses were observed in the sediment plus ethanol or water only control groups, but the solvent concentration used was not provided.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Frog eggs were collected from a pond in southern Sweden. Eggs were 2-3 days old. It was stated that the ponds received "organic contaminants only from airborne fallout" but no data was given to support this statement. Initial DEHP concentration in frog eggs was not determined.
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<b>Study Citation:</b>	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5508563			
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 characterize toxicological effects. There were approximately 100 eggs in each test vessel and 5 replicates were used for each of the eight concentrations tested and the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The housing and environmental conditions were reported and seemed to be conducive to health. The study was conducted at 5oC on 12 h light:12 h dark photoperiod.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest. 20-25 tadpoles were collected from each test vessel, homogenized, extracted with solvents, and analyzed using capillary gas chromatography.
	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 sampled at the end of the experiment to determine tissue concentration of DEHP.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. The experiment ran for 60 days, and no information on water quality was provided.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	Low	The concentrations in the tadpoles were shown in a figure. The actual concentration values are hard to interpret from the figure and in text. Only a range of values was given across all the treatment groups. The data was presented without measures of variability.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5508563			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was only identified by the chemical name. No other information was provided.
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Kebo-Grave (Sweden), but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as 97%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	The biological response of the negative control groups were adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental set up and test media preparation methods were reported but did not completely account for physical-chemical properties.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using capillary gas chromatography. Measured concentrations were similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 8 treatment groups and two controls (sediment plus ethanol and water only). The number of exposure groups and spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	Medium	No effects on biological responses were observed in the sediment plus ethanol or water only control groups, but the solvent concentration used was not provided.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Frog eggs were collected from a pond in southern Sweden. Eggs were 2-3 days old. It was stated that the ponds received "organic contaminants only from airborne fallout" but no data was given to support this statement. Initial DEHP concentration in frog eggs was not determined.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
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<b>Study Citation:</b>	Larson, P., Thuren, A. (1987). D-2-ethylhexylphthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. Environmental Toxicology and Chemistry 6(6):417-422.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana arvalis</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5508563			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. There were approximately 100 eggs in each test vessel and 5 replicates were used for each of the eight concentrations tested and the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The housing and environmental conditions were reported and seemed to be conducive to health. The study was conducted at 5oC on 12 h light:12 h dark photoperiod.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. The hatching success of eggs were determined by counting tadpoles at 5, 12 and 30 days after exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Descriptive statistics results (mean and standard deviation) were presented, but statistical tests to find significant differences among treatment groups were not conducted.
	Metric 22:	Reporting of Data	Medium	Percent hatch of the moor frog eggs were presented for each treatment and control group via a figure. The counts of tadpoles recorded at 5, 12 and 30 days were not provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Mortality outcome for percent hatch was presented in Fig 1, and tadpole survival was reported in the results section.			

**Overall Quality Determination****Medium**

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

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. There were approximately 100 eggs in each test vessel and 5 replicates were used for each of the eight concentrations tested and the controls.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The housing and environmental conditions were reported and seemed to be conducive to health. The study was conducted at 5oC on 12 h light:12 h dark photoperiod.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. The hatching success of eggs were determined by counting tadpoles at 5, 12 and 30 days after exposure.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: 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 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Descriptive statistics results (mean and standard deviation) were presented, but statistical tests to find significant differences among treatment groups were not conducted.
	Metric 22: Reporting of Data	Medium	Percent hatch of the moor frog eggs were presented for each treatment and control group via a figure. The counts of tadpoles recorded at 5, 12 and 30 days were not provided.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The tadpole counts recorded at 5, 12 and 30 days were not provided. The percent hatch of the frog eggs were shown via a figure.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in <i>Rana chensinensis</i> tadpoles. <i>Environmental Toxicology</i> 33(1):112-121.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana chensinensis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Receptor binding/ regulation of receptor activity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493510			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	DEHP was identified as the test substance by name only; no structure or CASN were reported.	
Metric 2:	Test Substance Source	Low	DEHP was obtained from Sigma Aldrich Corporation. It was not analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	High	Purity was 99% as reported from the manufacturer.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A negative control was used and replicated.	
Metric 5:	Negative Control Response	High	Control responses were adequate and are reported within figure 6 on page 8/10.	
Metric 6:	Randomized Allocation	Medium	Allocation of tadpoles was random.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	Concentrations were not measured during the study. Water was refreshed 50% every day and 100% every 3 days.	
Metric 8:	Consistency of Exposure Administration	Medium	Exposures appear consistent, but without measured concentrations, there is some uncertainty.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and the solubility of DEHP and loss of the test substance may have been an issue. DEHP is poorly soluble in water and no solvent was used.	
Metric 10:	Exposure Duration and Frequency	High	Exposure was conducted until amphibians underwent metamorphosis, and days to metamorphosis was calculated.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Treatments were spaced adequately to capture effects to metamorphosis: 0, 0.1, 1 and 10 umol/L DEHP.	
Metric 12:	Testing at or Below Solubility Limit	Medium	Concentrations did not exceed the water solubility limit, but the test substance may also sorb to test vessels and concentrations were not measured.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Medium	No captive source was used, wild breeding pairs of frogs were collected from China, and the tadpoles produced by those breeding pairs were used for the experiment.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions and animal husbandry are described and were comparable.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	30 tadpoles per tank and 4 tanks per treatment were used. For hormone measurements and gene expression, n=5 replicates per treatment were used.	

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<b>Study Citation:</b>	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in <i>Rana chensinensis</i> tadpoles. <i>Environmental Toxicology</i> 33(1):112-121.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana chensinensis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Receptor binding/ regulation of receptor activity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493510			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	No water quality data was reported.	
	Metric 17: Outcome Assessment Methodology	High	Mechanistic endpoints evaluated in this form included: Thyroid hormone concentrations (T3/T4) and gene expression of sex steroid relevant genes.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistics are clearly described, and sufficient data were provided to conduct an independent statistical analysis.	
	Metric 22: Reporting of Data	High	T4 and T3 concentration are reported for treatments and control in Figure 4. Gene expression is reported in Figure 6.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes described.	
Additional Comments:	Study examined the effects of DEHP on amphibian metamorphosis. Several endpoints relevant to the endocrine system (which controls development/metamorphosis) were measured. Mechanistic endpoints evaluated in this form included: Thyroid hormone concentrations (T3/T4) and gene expression of sex steroid relevant genes. Concentrations are nominal and were not verified. DEHP concentration may have been significantly lower than nominal if sorption to the test vessels occurred or if there were issues with the solubility. Other experiments have shown loss of the test substance over time.			

**Overall Quality Determination****Medium**

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

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<b>Study Citation:</b>	Zhang, Y., Li, X., Gao, J., Wang, H. (2018). Influence of DEHP on thyroid, sex steroid-related genes and gonadal differentiation in <i>Rana chensinensis</i> tadpoles. <i>Environmental Toxicology</i> 33(1):112-121.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Rana chensinensis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493510			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	No water quality data was reported.
	Metric 17:	Outcome Assessment Methodology	High	Sensitive endpoints were used that adequately captured effects to development and growth. The endpoints included: Body weight, total length, hind limb length, and T0.5 (the time necessary for 50% of the tadpoles to reach Gosner stage 42 which is peak metamorphosis).
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and 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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics are clearly described, and sufficient data were provided to conduct an independent statistical analysis.
	Metric 22:	Reporting of Data	High	Developmental/growth measurements for treatments and control are reported in Table 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes described.
Additional Comments:	Study examined the effects of DEHP on amphibian metamorphosis. Endpoints relevant to development and growth included: Length of the hind limb, total length, body length, and T05 (the number of days for 50% of tadpoles to reach peak metamorphosis).Concentrations are nominal and were not verified. DEHP concentration may have been significantly lower than nominal if sorption to the test vessels occurred or if there were issues with the solubility. Other experiments have shown loss of the test substance over time.			
Overall Quality Determination		Medium		

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

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

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Adult			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].	
Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."	
Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
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				
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	Details of exposure administration were not reported.	
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 the study type.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one exposure dose was used.	
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Medium	There are uncertainties about the age of test organisms.	
Metric 14:	Acclimatization and Pretreatment Conditions	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 and replicates were not reported.	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.	

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<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdnerii</i> ; Adult
<b>Health Outcome:</b>	Nutritional & Metabolic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5353221

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
	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.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group. Results were also described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation was for lipid content.

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].	
	Metric 2: Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	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				
	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	Details of exposure administration were not reported.	
	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 the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one exposure dose was used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are uncertainties about the age of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 and replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
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<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdnerii</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	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, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None				
<b>Overall Quality Determination</b>			<b>Low</b>	

<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Liver toxicology			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].	
	Metric 2: Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	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				
	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	Details of exposure administration were not reported.	
	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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one exposure dose was used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are uncertainties about the age of the test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 and replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
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<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdnerii</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Liver toxicology
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5353221

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group. Results were also described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This evaluation was for enzymes.		

**Overall Quality Determination****Low**

<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Adult			
<b>Health Outcome:</b>	Hepatic/Liver			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by accepted name [Di-(2-ethylhexyl)phthalate (DEHP)].
	Metric 2:	Test Substance Source	High	The test substance source was identified: "Di-(2-ethylhexyl)phthalate (DEHP) was purchased fromKoch-Light Laboratories Ltd. (Colnbrook, UK)."
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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				
	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	Details of exposure administration were not reported.
	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 the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one exposure dose was used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There are uncertainties about the age of the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	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 and replicates were not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system included few details.
	Metric 17:	Outcome Assessment Methodology	Low	The methods used to assess liver weights were not reported.
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<b>Study Citation:</b>	Henderson, R. J., Sargent, J. R. (1983). Studies on the effects of di-(2-ethylhexyl) phthalate on lipid metabolism in rainbow trout ( <i>Salmo gairdnerii</i> ) fed zooplankton rich in wax esters. <i>Comparative Biochemistry and Physiology - Part C: Comparative Pharmacology</i> 74(2):325-330.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdnerii</i> ; Adult			
<b>Health Outcome:</b>	Hepatic/Liver			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5353221			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Low	Since methods were not reported, it is uncertain whether there was consistency.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Liver weight data was shown in Table 1, and results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and measures of variability were shown with the data.
Additional Comments: This evaluation is for the liver weight assessment.				
<b>Overall Quality Determination</b>			<b>Low</b>	

<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	791717			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was unclear if the author used a negative control or a solvent control.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Researchers used radiolabeled chemical to monitor concentrations.	
	Metric 10: Exposure Duration and Frequency	High	A flow-through design with an appropriate duration was reported.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.	
Domain 4: Test Organism				
	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	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 (3) were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.	

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<b>Study Citation:</b>	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	791717

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	791717			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was unclear if the author used a negative control or a solvent control.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Researchers used radiolabeled chemical to monitor concentrations by GLC.	
	Metric 10: Exposure Duration and Frequency	High	A flow-through design with an appropriate duration were reported.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.	
Domain 4: Test Organism				
	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	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 (3) were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	791717

Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was not conducted, which is typical for BCF assessments.
Metric 22:	Reporting of Data	Low	Data were only reported for some outcomes, not for controls.
Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Mehrlle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	791717			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was unclear if the author used a negative or a solvent control.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Researchers used radiolabeled chemical to monitor concentrations.	
	Metric 10: Exposure Duration and Frequency	High	A flow-through design with an appropriate duration was reported.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit and used acetone to aid solubility.	
Domain 4: Test Organism				
	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	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 (3) were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Organism environmental conditions were conducive to the maintenance of health, though few details were provided.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	Mehrle, P. M., Mayer, F. L. (1976). Di-2-ethylhexyl phthalate: Residue dynamics and biological effects in rainbow trout and fathead minnows. Trace Substances in Environmental Health 10:519-524.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	791717

Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.
Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation form was added for development/growth endpoint with rainbow trout embryos.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo mykiss</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Test substance nomenclature was reported without CASRN.	
	Metric 2: Test Substance Source	Low	The DEHP was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number not provided. No analytical data was reported.	
	Metric 3: Test Substance Purity	High	At least 95% purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was reported.	
	Metric 5: Negative Control Response	High	The control response was acceptable.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, the headspace or the measures taken to prevent volatilization were not reported.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration was consistent across groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Sample extracts were analyzed by gas chromatography at the start and end of the test.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.	
	Metric 12: Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.	
	Metric 17: Outcome Assessment Methodology	High	Intended outcomes were reported.	

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo mykiss</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The environmental conditions were consistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis				
	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**

<b>Study Citation:</b>	Norrgrén, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). <i>Aquatic Ecosystem Health and Management</i> 2(3):311-317.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Juvenile			
<b>Health Outcome:</b>	Endocrine			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	No CAS or structure was provided. Chemical identified by nomenclature.
	Metric 2:	Test Substance Source	Low	Source as listed but not verified analytically.
	Metric 3:	Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Positive (estradiol in peanut oil) and carrier (peanut oil) controls were used.
	Metric 5:	Negative Control Response	High	The control responses in presence/absence of VTG in plasma are reported in fig 2 (Page 6/8).
	Metric 6:	Randomized Allocation	Low	random allocation was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors adequately reported preparation of injections and handling of fish. No survival metrics are reported for the 17-day study.
	Metric 8:	Consistency of Exposure Administration	Low	Authors indicate that injections per day were between 2-4 and also report two different durations for exposures (14 and 17 days).
	Metric 9:	Measurement of Test Substance Concentration	Low	Values are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	Medium	A 17 day exposure with daily administration of the test compound could result in VTG production.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	No justification was provided for the injection concentrations (80, 160 mg/kg, DEHP).
	Metric 12:	Testing at or Below Solubility Limit	N/A	Injections.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source and age of larvae were reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems with 50L per 5 animals (7.5 g each).
	Metric 15:	Number of Organisms and Replicates per Group	Low	These injections were conducted with triplicate groups (N=14)
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.

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<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). Aquatic Ecosystem Health and Management 2(3):311-317.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Juvenile			
<b>Health Outcome:</b>	Endocrine			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Uninformative	VTG was not detected from fish administered 80, 160 mg/kg DEHP daily for 17 days.
	Metric 18:	Consistency of Outcome Assessment	High	They outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	This study relied on the presence/absence of VTG on western blots.
	Metric 22:	Reporting of Data	Medium	This study relied on the presence/absence of VTG on western blots.
	Metric 23:	Explanation of Unexpected Outcomes	N/A	This study relied on the presence/absence of VTG on western blots.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Norrgrén, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). <i>Aquatic Ecosystem Health and Management</i> 2(3):311-317.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.	
	Metric 2: Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but the DEHP was not verified analytically.	
	Metric 3: Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Positive (estradiol in peanut oil) and carrier (peanut oil) controls were used.	
	Metric 5: Negative Control Response	High	The control responses in the presence/absence of VTG in plasma were reported and adequate (Figure 2, Page 6/8).	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Authors adequately reported preparation of injections and handling of fish. There was a minor omission on whether handling alone due to intraperitoneal injection administration caused any unwarranted stress or morbidity and/or mortality in fish.	
	Metric 8: Consistency of Exposure Administration	Low	Authors indicate that injections per day were between 2-4, and also report two different durations for exposures (14 and 17 days).	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP concentrations in the intraperitoneal injection preparations were not measured. DEHP values in intraperitoneal injection (80 and 160 mg/kg body weight) were only reported as nominal.	
	Metric 10: Exposure Duration and Frequency	Medium	A 17 day exposure with daily administration of the test compound could result in vitellogenin (VTG) production. However, it is not clear how many injections were administered since the authors reported that organisms were injected intraperitoneally with test solution 2-4 times during the 17-day exposure period. The authors did not explain why they administered 2-4 injections.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were 80 and 160 mg DEHP/kg body weight as well as controls. However, no justification was provided for the injection concentrations.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via injection.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source and age of the juveniles were reported. The species ( <i>Salmo salar</i> ) was appropriate for the intended outcome of the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate for one week to the rearing conditions in a flow-through (river water provided) system with 50L per 5 animals (7.5 g each).	

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<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). Aquatic Ecosystem Health and Management 2(3):311-317.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 fish per group were administered the intraperitoneal injections. The 5 fish per group were maintained in separate tanks. There were no replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (plasma collection and western blot analysis of vitellogenin) was reported and adequate for the outcome of interest (determine the concentrations of vitellogening in plasma). While vitellogenin was detected in the positive control group, it was not detected in the DEHP (80 and 160 mg/kg body weight) groups.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	This study relied on the presence/absence of a vitellogenin band on western blots.
	Metric 22:	Reporting of Data	Medium	This study relied on the presence/absence of vitellogenin on western blots. In the results section, the authors described that vitellogenin was not detected in plasma after intraperitoneal injections of DEHP.
	Metric 23:	Explanation of Unexpected Outcomes	High	In the results section, the authors described that vitellogenin was not detected in plasma after intraperitoneal injections of DEHP. The authors thought that the findings were surprising since estrogenic chemicals such as DEHP had been shown in previous investigations to cause proliferation of MCF-7 cells in vitro. Moreover, the authors explained that the size and dose regime of the experiment may have affected the production of vitellogening and indicated that an alternative biomarker used for the detection of estrogenic chemicals such as DEHP could be the production of zona radiata protein (ZRP).
Additional Comments:	The aim of the present study was to perform in vivo studies on Atlantic salmon to evaluate the effects of DEHP exposure (oral/dietary or intraperitoneal) to study feminization in terms of skewed sex ratios and vitellogenin (VTG) production. Because VTG is stored in the liver, liver development (liver somatic index) was also examined. This form was used to evaluate the effect of DEHP exposure by intraperitoneal injection on the presence of VTG in the plasma.			

**Overall Quality Determination****Medium**

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

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The methods assessment was appropriate for the outcomes reported (Hepato somatic index).
	Metric 18: Consistency of Outcome Assessment	High	They outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20: Outcomes Unrelated to Exposure	Low	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	ANOVA was reported for determining significant differences in HSI.
	Metric 22: Reporting of Data	Medium	The sample number, HSI value and SD are presented in Table 2 (Page 5/8).
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variance was presented in Table 2. The sample sizes are not equal between treatment and control groups.

Additional Comments: None

**Overall Quality Determination****Medium**

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

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<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). Aquatic Ecosystem Health and Management 2(3):311-317.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	They outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Chi-squared analysis was reported for determining significant differences in HSI.
	Metric 22:	Reporting of Data	Low	Figure 1 presented the number of females among treatments and control groups. The total number of animals represented was not reported for each group, which should have been reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The number of phenotypic females were presented but no other information was reported.
Additional Comments: None				
<b>Overall Quality Determination</b>			<b>Medium</b>	

<b>Study Citation:</b>	Norrgrén, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). <i>Aquatic Ecosystem Health and Management</i> 2(3):311-317.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.	
	Metric 2: Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but DEHP was not verified analytically.	
	Metric 3: Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.	
	Metric 5: Negative Control Response	High	The control responses in liver somatic index (LSI) of juvenile salmon were reported and adequate (Table 2, Page 5/8).	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Authors adequately reported preparation of the treated diet as well as handling of the compound in section 2.2 (page 3/8).	
	Metric 8: Consistency of Exposure Administration	High	There is no evidence suggesting that dietary treatments were not administered equally among groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP in experimental diet was not measured. DEHP values in diet (300 and 1500 mg/kg food) were reported as nominal and based on an estimated 2% body weight basis.	
	Metric 10: Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad differentiation in fry within Atlantic Salmon.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors reported exposing organisms to 300 and 1500 mg DEHP/kg of food. However, the authors presented no justification for the concentrations used in the feeding study.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposures were via dietary treatments.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source and age of larvae were reported. The species ( <i>Salmo salar</i> ) was appropriate for the intended outcome of the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.	
	Metric 15: Number of Organisms and Replicates per Group	Low	No replication was reported but each treatment had 200 individuals.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). <i>Aquatic Ecosystem Health and Management</i> 2(3):311-317.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology [liver dissection and weighing to calculate the liver somatic index (LSI): liver weight/total body weight X 100] was appropriate for the outcome reported (DEHP effect on liver development/growth).
	Metric 18:	Consistency of Outcome Assessment	High	They outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented to indicate that differences were due to attrition or animal health.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	One-way analysis of variance (ANOVA) was used to determine if there was a difference in the liver somatic index (LSI) between the control group and the exposed groups.
	Metric 22:	Reporting of Data	High	The sample size, mean LSI value and standard deviation across treatment groups were presented in Table 2 (Page 5/8).
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variance was presented in Table 2. The sample sizes are not equal between treatment and control groups.
<b>Additional Comments:</b>	The aim of the present study was to perform in vivo studies on Atlantic salmon to evaluate the effects of DEHP exposure (oral/dietary or intraperitoneal) to study feminization in terms of skewed sex ratios and vitellogenin (VTG) production. Because VTG is stored in the liver, liver development (liver somatic index) was also examined. This form was used to evaluate liver developmental/growth effects of DEHP dietary exposure in salmon fry.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). Aquatic Ecosystem Health and Management 2(3):311-317.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	No CAS or structure was provided. The chemical was only identified by nomenclature.	
	Metric 2: Test Substance Source	Low	The chemical source was reported (Neste-oxo, Stenungsund, Sweden), but DEHP was not verified analytically.	
	Metric 3: Test Substance Purity	High	Purity from the manufacturer was reported as 99.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Positive (estradiol) and solvent (ethanol) controls were used.	
	Metric 5: Negative Control Response	High	The control responses (positive and solvent control) for sex determination were adequate and reported in Figure 1 (Page 5/8).	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Authors adequately reported preparation of the treated diet as well as handling of the compound in section 2.2 (page 3/8).	
	Metric 8: Consistency of Exposure Administration	High	There is no evidence suggesting that dietary treatments were not administered equally among groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP in experimental diet was not measured. DEHP values in diet (300 and 1500 mg/kg food) were reported as nominal and based on an estimated 2% body weight basis.	
	Metric 10: Exposure Duration and Frequency	High	4 weeks of treatment diet should cover the period of gonad development and differentiation within Atlantic Salmon.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The authors reported exposing organisms to 300 and 1500 mg DEHP/kg of food. However, the authors presented no justification for the concentrations used in the feeding study.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposures were via dietary treatments.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source and age of larvae were reported. The species ( <i>Salmo salar</i> ) was appropriate for the intended outcome of the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Animals were allowed to acclimate to flow-through (river water provided) systems for 4 weeks before the 4 week feeding period.	
	Metric 15: Number of Organisms and Replicates per Group	Low	No replication was reported, but each treatment had 200 individuals.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Norrgren, L., Blom, A., Andersson, P. L., Boerjeson, H., Larsson, J., D.G., Olsson, P. E. (1999). Effects of potential xenoestrogens (DEHP, nonylphenol and PCB) on sexual differentiation in juvenile Atlantic salmon ( <i>Salmo salar</i> ). Aquatic Ecosystem Health and Management 2(3):311-317.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo Salar</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5646979			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Housing was adequate, however, the authors did not provide or record environmental parameters during the study.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology (gonad classification as males those with thin colorless gonads and as females those with pinkish 'bulbs'; sex was confirmed by light microscopic examination) was reported and appropriate for the intended outcome of interested (DEHP effects on sex determination).
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appeared to be assessed similarly across the treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Nothing was reported to indicate that environmental variables confounded the test results, however, the authors did not report these conditions for the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was presented to indicate that differences were due to attrition or animal health. Authors did not report survival.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A chi-square test for association was used to determine if there was a correlation between the number of females in each group and diet.
	Metric 22:	Reporting of Data	Low	Figure 1 presented the number of females among treatment and control groups. The total number of organisms (males plus females) per treatment group should have been reported. The authors only reported a range of the total number of individuals per treatment group to be between 82 and 184, which a wide range and it is impossible to determine the actual ratio of males to females.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The number of phenotypic females were presented but no other information was reported.
<b>Additional Comments:</b>	The aim of the present study was to perform in vivo studies on Atlantic salmon to evaluate the effects of DEHP exposure (oral/dietary or intraperitoneal) to study feminization in terms of skewed sex ratios and vitellogenin (VTG) production. Because VTG is stored in the liver, liver development (liver somatic index) was also examined. This form was used to evaluate DEHP dietary exposure on the gonad development in salmon fry. This form also assesses the sex ratio outcome displayed in Fig. 1.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

<b>Study Citation:</b>	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon ( <i>Salmo salar</i> ) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo salar</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5678430			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No other information was provided.	
Metric 2:	Test Substance Source	Low	The source was reported as Neste-Oxo AB, Sweden, but the test substance identity was NOT analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).	
Metric 5:	Negative Control Response	High	The biological response of the control was 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	High	The experimental system and methods for preparation of the test media were described in adequate detail. Concentration of the test substance in the diet was measured before the initiation of the experiment.	
Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using GC-MS.	
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest. Fish were fed DEHP dosed diets for 4 weeks and were fed (approximately 2% of their body weight daily) four times per day.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.	
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.	
Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that there were 1000 animals in each experimental group. Replicates were not reported.	
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<b>Study Citation:</b>	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon ( <i>Salmo salar</i> ) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo salar</i> ; Larvae		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5678430		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during the exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group. The histopathology of gonads was conducted 4 months post -exposure. Housing and environmental conditions during this post-exposure period were not mentioned.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (histological evaluation of gonads) addressed or reported the intended outcome(s) of interest.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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. The mortality in all groups was low- 3-5% at 4 months post exposure.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was performed and described well. Sex ratios, intersex ratios, and precocious ratios were analyzed using Fisher's exact test for significant differences between groups.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Fig 2). Pictures of histopathology results from control fish and fish exposed to the highest DEHP concentration were provided (Figures 3-5). Numbers of fish presenting ovo-testis (intersex) were counted for each treatment.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The histological evaluation included gonadal differentiation and presence of ovo-testis. Findings were supported by data and photos of histological evaluations.		
<b>Overall Quality Determination</b>		<b>High</b>	

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

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

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Norman, A., Börjeson, H., David, F., Tienpont, B., Norrgren, L. (2007). Studies of uptake, elimination, and late effects in atlantic salmon ( <i>Salmo salar</i> ) dietary exposed to di-2-ethylhexyl phthalate (DEHP) during early life. Archives of Environmental Contamination and Toxicology 52(2):235-242.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Salmo salar</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5678430			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Housing, environmental conditions, food, and biomass loadings during exposure seemed to be conducive for the maintenance of health. No significant mortality was observed in any treatment group.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: 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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for fish tissue concentrations.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 1).
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) for fish tissue concentrations.
Additional Comments:	Fish tissue concentration values were provided on a wet-weight and lipid normalized basis. Also, fish tissue concentrations of mono-2-ethyl hexyl phthalate (MEHP), a metabolite of DEHP was also reported in the paper.			
Overall Quality Determination		High		

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

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

**Overall Quality Determination****Uninformative**

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

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

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	DumPERT, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxi-cology and Environmental Safety</i> 8(1):55-74.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	31448			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms that were exposed to DEHP per basin was not clear. Au-thors state a total of 360 larvae hatched from 4 basins and then state 30 tadpoles per basin remained.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Authors do not report pH, DOC, or DO for water in test basins. Authors state loading 6 L basins with 200 eggs per basin with low hatching success.	
	Metric 17: Outcome Assessment Methodology	Low	Details for assessing survival were not described (unclear whether dead larvae were removed daily or on a regular basis).	
	Metric 18: Consistency of Outcome Assessment	Low	Timing and frequency of the execution of outcome assessment protocol were not re-ported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There was high attrition of larvae from eggs prior to initiating the experiment, and it was not reported in which tanks the attrition was the greatest.	
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors state high attrition of test organisms prior to conducting the experiment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	This was a limit test.	
	Metric 22: Reporting of Data	High	Survival rate after 200 days was shown in Figure 2.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Authors did not report variability.	
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Dumpert, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxicology and Environmental Safety</i> 8(1):55-74.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	31448			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure was provided in Figure 1. The source was not reported. The purity was not reported.
	Metric 2:	Test Substance Source	Low	
	Metric 3:	Test Substance Purity	Low	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a Holtfreter solution control and a water-only control were utilized in this experiment. Time from egg to full developed frog is stated in Table 2 (variation among test organisms within the control basins was not provided). Random allocation was not stated.
	Metric 5:	Negative Control Response	Medium	
	Metric 6:	Randomized Allocation	Low	
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Authors utilized components in the experimental set-up most likely containing DEHP (Plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, preparation of the stock solutions of DEHP was not adequately described. Concentrations were not reported for Experiment 2. Details of DEHP dosing and renewal of the basins were limited.
	Metric 8:	Consistency of Exposure Administration	Low	
	Metric 9:	Measurement of Test Substance Concentration	Low	
	Metric 10:	Exposure Duration and Frequency	Low	DEHP concentrations in water were not reported for Experiment 2. DEHP concentration differences between the control basin and DEHP basins were provided, but concentrations of the basins were not provided. The exposure duration lasted for the duration of development (time to metamorphosis), and was thus different across treatments. DEHP (10 ppm) was added either once or at repeated intervals in the DEHP basins.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	
	Metric 12:	Testing at or Below Solubility Limit	Low	
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source of breeding stock was described. The acclimatization period was not described by authors (unclear when dosing commenced).
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	
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<b>Study Citation:</b>	DumPERT, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxicology and Environmental Safety</i> 8(1):55-74.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	31448			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms that were exposed to DEHP per basin was not clear. Authors state a total of 360 larvae hatched from 4 basins and then state 30 tadpoles per basin remained.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Authors do not report pH, DOC, or DO for water in test basins. Authors state loading 6 L basins with 200 eggs per basin with low hatching success.	
	Metric 17: Outcome Assessment Methodology	Medium	Authors cite Nieuwkoop and Faber 1956 for outcome assessment methodology. Details of the histological examination were not well described.	
	Metric 18: Consistency of Outcome Assessment	Low	The timing and frequency of the execution of outcome assessment protocol were not reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There was high attrition of larvae from eggs, and it was not reported in which tanks the attrition was the greatest.	
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors state high attrition of test organisms prior to conducting the experiment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Uninformative	No statistics were performed for data in Table 2.	
	Metric 22: Reporting of Data	High	Time to development was shown in Figure 2.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Authors did not report variability.	
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	DumPERT, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxi-cology and Environmental Safety</i> 8(1):55-74.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	31448			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified as Di(2-ethylhexyl) phthalate (DEHP), and the structure was provided in Figure 1.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent-control and a water-only control were utilized in this experiment.
	Metric 5:	Negative Control Response	Uninformative	In Experiment I, the methyl alcohol control was observed to cause embryo toxicity (perhaps by promoting the growth of bacteria), thus making it impossible to differentiate any effects due to DEHP alone. Experiment II addressed these concerns by removing the use of the alcohol control.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Authors utilized components in the experimental set-up most likely containing DEHP (plastic tubing) as shown in Table 1 (control basins) and in the text. Additionally, prepa-ration of the stock solutions of DEHP was not adequately described.
	Metric 8:	Consistency of Exposure Administration	Low	Details of DEHP dosing and renewal of the basins were limited.
	Metric 9:	Measurement of Test Substance Concentration	Medium	DEHP concentrations in water were measured via GC-FID and were measured imme-diately after dosing and 12 days after dosing. Di-n-hexyl phthalate was utilized as a standard solution. No details were provided on reproducibility or recovery.
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was not explicitly stated, but as is common in amphibian devel-opmental studies, lasted for the duration of development (or time to complete metamor-phosis).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five exposure concentrations ranged from 0.1-20 ppm DEHP. One basin containing between 70-83 larvae was utilized per test concentration and per control group.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Reported solubility for DEHP is approximately 0.3 ppm. Authors report test concen-trations above this limit, but they report utilizing methanol as the solvent to increase solubility of DEHP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source of breeding stock was described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The acclimatization period was not described by authors (unclear when dosing com-menced).

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<b>Study Citation:</b>	DumPERT, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxicology and Environmental Safety</i> 8(1):55-74.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	31448			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	There was one basin per test concentration containing between 70-83 larvae per basin.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Authors do not report pH, DOC, or DO for water in test basins.	
	Metric 17: Outcome Assessment Methodology	Medium	Authors cite Nieuwkoop and Faber 1956 for outcome assessment methodology. Details of the histological examination were not well described.	
	Metric 18: Consistency of Outcome Assessment	Low	Timing and frequency of the execution of outcome assessment protocol were not reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Uninformative	Bacterial infection in the solvent control and the DEHP control basin but not in the water control basin confounded interpretation of developmental endpoints from this experiment.	
	Metric 20: Outcomes Unrelated to Exposure	Uninformative	Authors report the presence of bacterial infection in the solvent control and all DEHP treatment basins, and state they could not distinguish whether embryotoxic effects were due to DEHP treatment or presence of bacteria in the basins.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Uninformative	Statistics were not reported for this data.	
	Metric 22: Reporting of Data	Uninformative	Developmental endpoint data were not reported for each treatment group, nor were they distinguished between solvent control and DEHP treatments.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Authors did not report variability.	
Additional Comments:	In Experiment I, the methyl alcohol control was observed to cause embryo toxicity (perhaps by promoting the growth of bacteria), thus making it impossible to differentiate any effects due to DEHP alone.			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	DumPERT, K., Zietz, E. (1984). Platanna ( <i>Xenopus laevis</i> ) as a test organism for determining the embryotoxic effects of environmental chemicals. <i>Ecotoxicology and Environmental Safety</i> 8(1):55-74.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Xenopus laevis</i> ; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	31448		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Authors do not report pH, DOC, or DO for water in test basins. The low level of survival in all groups indicate that 6 L may not be an adequate size for the number of embryos per basin.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not described (i.e. were dead embryos checked for daily and removed from basins). The endpoint was described as survival, not mortality.
Metric 18:	Consistency of Outcome Assessment	Low	The timing and frequency of the execution of outcome assessment protocol were not reported. Authors stated all animals were euthanized at 3 months after hatching, but the determination of mortality prior to this point was not described.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Uninformative	Bacterial infection in the solvent control and the DEHP control basin but not in the water control basin could confound interpretation of mortality in this study.
Metric 20:	Outcomes Unrelated to Exposure	Uninformative	Authors report the presence of bacterial infection in the solvent control and in all the DEHP treatment basins, and they state they could not distinguish whether embryotoxic effects were due to DEHP treatment or the presence of bacteria in the basins.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistics were not reported for this data.
Metric 22:	Reporting of Data	Uninformative	Survival data were not reported for each treatment group but instead was presented as an average across all DEHP groups.
Metric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report variability.
Additional Comments:	In Experiment I, the methyl alcohol control was observed to cause embryo toxicity (perhaps by promoting the growth of bacteria), thus making it impossible to differentiate any effects due to DEHP alone.		

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Woin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination and Toxicology 38(2):220-225.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Aeshna sp.</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	790132			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not addressed.
	Metric 18:	Consistency of Outcome Assessment	Low	Details of the outcome assessment protocol were not reported.
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	Uninformative	Statistical analysis was not performed.
	Metric 22:	Reporting of Data	Low	It was unclear which sediment concentration was system 1 or system 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Woin, P., Larsson, P. (1987). Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. Bulletin of Environmental Contamination and Toxicology 38(2):220-225.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Aeshna sp.</i> ; Larvae
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	790132

Domain	Metric	Rating	Comments
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 analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	It was unclear which sediment concentration was system 1 or system 2.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Asellus breviicaudus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 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	Low	Few 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.
	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	Only one treatment was reported.
	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 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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently detailed to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Asellus breviicaudus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.
Domain 6: Confounding / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	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 Quality Determination****Uninformative**

<b>Study Citation:</b>	Cruciani, V., Iovine, C., Thomé, J. P., Joaquim-Justo, C. (2015). Impact of three phthalate esters on the sexual reproduction of the Monogonont rotifer, <i>Brachionus calyciflorus</i> . <i>Ecotoxicology</i> 25(1):192-200.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Brachionus calyciflorus</i> ; Pallas; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070931			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The test substance was identified only by the chemical name. No other information was provided.	
Metric 2:	Test Substance Source	Low	The test substance was obtained from Sigma Aldrich, Germany, but the test substance identity was not analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure). Both water and solvent controls were used.	
Metric 5:	Negative Control Response	High	The biological responses of the controls were reported and were adequate.	
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
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 exposure. Concentrations of the test substance were not measured during the study.	
Metric 8:	Consistency of Exposure Administration	Medium	Details of the initial exposure administration were reported, but details of the exposure administration past 48 hours were not clearly reported. It was reported that at 48 hours, rotifers were transferred to new test media, and at 48, 72 and 96 hours, tubes were emptied into a glass petri dish for counting males and females. No details were given on how the experiment was continued until 96 hours.	
Metric 9:	Measurement of Test Substance 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 and/or outcomes of interest.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 4 exposure groups for DEHP, and 5 replicates were run for each concentration.	
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 Organism				
Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	

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<b>Study Citation:</b>	Cruciani, V., Iovine, C., Thomé, J. P., Joaquim-Justo, C. (2015). Impact of three phthalate esters on the sexual reproduction of the Monogonont rotifer, <i>Brachionus calyciflorus</i> . <i>Ecotoxicology</i> 25(1):192-200.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Brachionus calyciflorus</i> ; Pallas; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070931			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, and food were conducive to the 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 fertilized female, and detached fertilized eggs. In the paper and in the cited reference (Preston et al. 2000), it was reported that test tube contents were emptied into a petri dish at 48 and 72 hours, but there were no details given regarding the methods for continuing the experiment until 96 hours.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Outcomes were assessed at 48, 72 and 96 hours in treatment groups and controls.
Domain 6: Confounding / 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 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 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:	DEHP exposure (0.05 to 2mg/L) caused no significant effect on population growth rate, mixis rate, fertilization rate, or resting egg production in rotifers.			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	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 <i>Brachionus calyciflorus</i> Pallas. <i>Aquatic Ecology</i> 43(2):395-402.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1336226			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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	Low	The number of exposure groups and the spacing of exposure levels were inadequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolution.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Eggs were collected in the sediments of Lake Jinghu and cultured in the lab. There are minor reservations regarding the source of test organisms that are unlikely to have a substantial impact on results.	
	Metric 14: Acclimatization and Pretreatment 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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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 <i>Brachionus calyciflorus</i> Pallas. <i>Aquatic Ecology</i> 43(2):395-402.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1336226

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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 <i>Brachionus calyciflorus</i> Pallas. <i>Aquatic Ecology</i> 43(2):395-402.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1336226			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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	Low	The number of exposure groups and the spacing of exposure levels were inadequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Low	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolution.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Eggs were collected in the field and cultured in the lab. There are minor reservations regarding the source of test organisms that are unlikely to have a substantial impact on results.	
	Metric 14: Acclimatization and Pretreatment 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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
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<b>Study Citation:</b>	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 <i>Brachionus calyciflorus</i> Pallas. <i>Aquatic Ecology</i> 43(2):395-402.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Brachionus calyciflorus</i> ; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was reported to be Monsanto Chemical Supply in St. Louis, MO. It was not reported if the DEHP was analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.	
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 3: Exposure Characterization				
	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	High	Study authors cited the Committee on Methods of Toxicity Tests with Aquatic Organisms, 1975 for the methods used in the acute toxicity tests.	
	Metric 9: Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported.	
	Metric 10: Exposure Duration and Frequency	Medium	The study duration was reported to be 48h. 96h tests are typical for midge acute toxicity tests. The test duration did not appear to have a significant effect on the outcome.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The study authors did not report the number of exposure groups or the spacing of the groups for the acute tests.	
	Metric 12: Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under 0.1mL/L in all test concentrations and controls.	
Domain 4: Test Organism				
	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.	
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<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms per test chamber and the number of replicates was not reported, though this may have been included in the citation for methodology.	
Domain 5: Outcome Assessment				
	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 / 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 in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Methods described by Litchfield and Wilcoxon 1949 were used to determine EC50 values.	
	Metric 22: Reporting of Data	Low	Only EC50 values were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 2.	
Additional Comments:	This portion of the evaluation was on the acute toxicity of DEHP on <i>C. pulmosus</i> . 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 Quality Determination****Uninformative**

<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The DEHP was identified by name only.
	Metric 2:	Test Substance Source	Low	The source of the 14C labelled DEHP was reported to be Pathfinders Laboratories Inc. in St. Louis, MO. It was not reported if the DEHP was analytically verified.
	Metric 3:	Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	This portion of the study did not report the use of a concurrent negative control.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control was not reported as there did not appear to be a negative control.
	Metric 6:	Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test media and concentrations. A diluter system was reported to be used for this portion of the study.
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. A diluter system was used to administer the test media, but the test chambers were not described. Test volumes were also not described.
	Metric 9:	Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported. Other sources were cited.
	Metric 10:	Exposure Duration and Frequency	High	It was reported there was a 4 day exposure with a 5 day period in which midges were in DEHP-free water.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level for this portion of the study, as the goal was not to have a dose response, but to observe any accumulation of DEHP in midge tissue.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from an in-house culture and the age of the organisms was appropriate.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report if the organisms were acclimated or needed to be acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms for this portion of the study was not reported, nor was the number of replicates.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. It was not reported if the midges were fed or what the biomass loading was.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–DEHP tissue accumulation–ADME.	
	Metric 18: Consistency of Outcome Assessment	Low	It was unclear how the DEHP in the midge tissue was determined.	
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 in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	There was only 1 treatment level and no control, so there was nothing to compare.	
	Metric 22: Reporting of Data	Low	The exposure response was reported in Figure 1, but there was not a control response reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	Variability was reported in Figure 1. The study authors did not report any unexpected outcomes.	
Additional Comments:	This portion of the evaluation was on the accumulation of 14C labelled DEHP in midge tissue. ADME was selected as the outcome of interest. This part of the evaluation received an unacceptable rating due to the lack of a negative control.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2: Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Ethanol was listed for solvent controls, however, authors report using ethanol concentrations at higher levels than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of the compounds.	
	Metric 5: Negative Control Response	Low	Control responses were not reported for the acute toxicity bioassays.	
	Metric 6: Randomized Allocation	Low	It was not reported how animals were allocated to treatment concentrations.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal with mortality assessed at 24 and 48 hours.	
	Metric 8: Consistency of Exposure Administration	High	Exposure administration appeared consistent among treatments and control.	
	Metric 9: Measurement of Test Substance Concentration	Low	The acute bioassay concentrations were not analyzed, and LC50 concentrations are reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration (48 hr) was appropriate.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	None of the treatment concentrations for the acute bioassays were reported. Range finding tests were not described.	
	Metric 12: Testing at or Below Solubility Limit	Low	Authors intentionally exceeded solubility concentrations for the acute bioassays. The range of concentrations was not reported, but LC50 values are above the solubility reported for this compound in the Final Scope (0.27 mg/L).	
Domain 4: Test Organism				
	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 Conditions	High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors listed 10 individuals per treatment concentration, but they did not report the level of replication for each treatment.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	DO, 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 / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	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		

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2: Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Ethanol was listed for solvent controls, however, authors report using ethanol concentrations at higher levels than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of the compounds.	
	Metric 5: Negative Control Response	Low	Control responses were not reported for acute toxicity bioassays.	
	Metric 6: Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal with mortality assessed at 24 and 48 hours.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and control.	
	Metric 9: Measurement of Test Substance Concentration	Low	The acute bioassay concentrations were not analyzed, and LC50 concentrations were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration (48 hr) is appropriate.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	None of the treatment concentrations for the acute bioassays were reported. Range finding tests were not described.	
	Metric 12: Testing at or Below Solubility Limit	Low	Authors intentionally exceeded solubility concentrations for the acute bioassays. The range of concentrations was not reported, but LC50 values were above the solubility reported for this compound in the Final Scope (0.27 mg/L).	
Domain 4: Test Organism				
	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 Conditions	High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors listed 10 individuals per treatment concentrations, but they did not report the level of replication for each treatment.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the acute bioassays.	
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<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The authors had a very detailed list of multiple criteria to determine mortality from the bioassays.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors used Litchfield and Wilcoxon method for LC50 estimation.
	Metric 22:	Reporting of Data	Medium	LC50 and 95% confidence intervals are reported, but results from each concentration are not available.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	None			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 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	Low	Few 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.
	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	Only one treatment was reported.
	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 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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.
Domain 6: Confounding / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	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 Quality Determination****Uninformative**

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2: Test Substance Source	Low	The source of radio labeled DEHP was listed as Pathfinder laboratories, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	N/A	For this ADME study, the authors do not use a control.	
	Metric 5: Negative Control Response	N/A	No control treatment within this ADME study.	
	Metric 6: Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The larval uptake assays were conducted as static non-renewal.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and control.	
	Metric 9: Measurement of Test Substance Concentration	High	GC was used to verify concentrations throughout the uptake study within the water, organisms, and substrate.	
	Metric 10: Exposure Duration and Frequency	High	120 hours is appropriate for this bioassay.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	The initial concentrations were chosen from the labs previous work on similar chemicals.	
	Metric 12: Testing at or Below Solubility Limit	High	The concentrations within this ADME study were at the reported solubility limit for DEHP (0.27 mg/l).	
Domain 4: Test Organism				
	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 Conditions	High	The pretreatment conditions were listed and similar to the uptake and elimination study (8 days).	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Samples sizes and procedures were appropriate for this ADME study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Water quality parameters were monitored for other studies within the manuscript but not reported for this experiment.	

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<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	High	The calculations of a quench curve for tissues was reported on page 30/62. Analytical methods for concentration determination of tissue, water, and substrate were also reported throughout time.	
	Metric 18: Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	These data were plotted (page 47/62), and elimination curves were created and presented with R values.	
	Metric 22: Reporting of Data	High	Data at all time points are presented in tables 15 and 16.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	None			
Overall Quality Determination		High		

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2: Test Substance Source	Low	The source of the radio labeled DEHP was listed as Pathfinder laboratories, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	N/A	For this ADME study, the authors do not use a control.	
	Metric 5: Negative Control Response	N/A	There was no control treatment within this ADME study.	
	Metric 6: Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The larval uptake assays were conducted as static non-renewal.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and controls.	
	Metric 9: Measurement of Test Substance Concentration	High	GC was used to verify concentrations throughout the uptake study within the water, organisms, and substrate.	
	Metric 10: Exposure Duration and Frequency	High	120 hours is appropriate for this bioassay.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	The initial concentrations were chosen from the labs previous work on similar chemicals.	
	Metric 12: Testing at or Below Solubility Limit	High	The concentrations within this ADME study were at the reported solubility limit for DEHP (0.27 mg/l).	
Domain 4: Test Organism				
	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 Conditions	High	The pretreatment conditions were listed and similar to the uptake and elimination study (8 days).	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Samples sizes and procedures were appropriate for this ADME study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Water quality parameters were monitored for other studies within the manuscript but not reported for this experiment.	
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<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	High	The calculations of a quench curve for tissues was reported on page 30/62. Analytical methods for concentration determination of tissue, water, and substrate were also reported throughout time.	
	Metric 18: Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	These data were plotted (page 47/62), and elimination curves were created and presented with R values.	
	Metric 22: Reporting of Data	High	Data at all time points are presented in tables 15 and 16.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	None			
Overall Quality Determination		High		

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was reported to be Monsanto Chemical Supply in St. Louis, MO. It was not reported if the DEHP was analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study reported the use of a concurrent negative control in which the solvent was used.	
	Metric 5: Negative Control Response	High	The biological response of the negative control was adequate and was reported in Table 4.	
	Metric 6: Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test media and concentrations. A diluter system was reported to be used for the chronic test.	
	Metric 8: Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. A diluter system was used to administer the test media, but the test chambers were not described. Test volumes were also not described. It is unclear at what point the F0 adults were mated for obtaining egg masses. It is unclear if the embryos were exposed or just monitored for hatch.	
	Metric 9: Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported. Other sources were cited.	
	Metric 10: Exposure Duration and Frequency	Uninformative	The duration for this portion of the chronic test was not reported. No more adults were reported to be emerging after 35 days, but it is unclear when the exposed F0 generation were mated or for how long the egg masses were exposed.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 3 reported exposure levels plus a control. More exposure levels or different spacing of the exposure levels may have yielded more in depth results.	
	Metric 12: Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under 0.1mL/L in all test concentrations and controls.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were obtained from an in-house culture and were the appropriate age for the study.	

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<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report if the organisms were acclimated or needed to be acclimated to test conditions.  Study authors reported using 100 organisms for the chronic test, but it was unclear how many replicates there were and how many organisms were in each exposure group.
	Metric 15:	Number of Organisms and Replicates per Group	Low	
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. Organisms were fed 0.12g of dog candy daily until pupation. It was not reported how many organisms were in each test chamber.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—egg mass production, number of eggs per mass, and hatchability of the eggs.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the outcome assessment were confusing and limited. It is unclear when the egg masses were counted and when the eggs were counted.
Domain 6: Confounding / 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 in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed for this portion of the study, but it was not described.
	Metric 22:	Reporting of Data	High	Control and exposure response data was reported in Table 4 and was adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments:	This portion of the evaluation was on the chronic toxicity of DEHP on midge reproduction. The number of egg masses produced and the number of eggs per egg mass were reported, so the reproductive outcome was selected. This part received an unacceptable rating due to the lack of information regarding the duration. It is unclear when the F0 adults were mated and when the eggs were counted. It appears as though the egg masses were then kept at the same exposure level as the F0 generation, so mortality we be the outcome of interest for the hatchability portion of the study.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was reported to be Monsanto Chemical Supply in St. Louis, MO. It was not reported if the DEHP was analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and the grade of the DEHP were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study reported the use of a concurrent negative control in which the solvent was used.	
	Metric 5: Negative Control Response	High	The biological response of the negative control was adequate and was reported in Table 3.	
	Metric 6: Randomized Allocation	Low	It was not reported how the larvae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test media and concentrations. A diluter system was reported to be used for the chronic test.	
	Metric 8: Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. A diluter system was used to administer the test media, but the test chambers were not described. Test volumes were also not described.	
	Metric 9: Measurement of Test Substance Concentration	Medium	It was reported that test concentrations were measured at the start of the test, but the methods used were not reported. Other sources were cited.	
	Metric 10: Exposure Duration and Frequency	Low	The duration was reported to be 2 days post when the last cast off pupal skin was found. According to Table 3, this was 35 days, though it was not explicitly stated anywhere in the article.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 3 reported exposure levels plus a control. More exposure levels or different spacing of the exposure levels may have yielded more in depth results.	
	Metric 12: Testing at or Below Solubility Limit	High	Study authors reported using an appropriate vehicle solvent, and it was kept under 0.1mL/L in all test concentrations and controls.	
Domain 4: Test Organism				
	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.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Study authors reported using 100 organisms for the chronic test, but it was unclear how many replicates there were and how many organisms were in each exposure group.	

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<b>Study Citation:</b>	Streufert, J. M., Jones, J. R., Sanders, H. O. (1980). Toxicity and biological effects of phthalate esters on midges ( <i>Chironomus plumosus</i> ). Transactions of the Missouri Academy of Science 14:33-40.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	813673; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	Medium	All tests were conducted at 22C with a 16L:8D photoperiod. Well water was used in the test media. Organisms were fed 0.12g of dog candy daily until pupation. It was not reported how many organisms were in each test chamber.	
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—adult emergence—development/growth.	
Metric 18:	Consistency of Outcome Assessment	High	Pupal skin cast offs were counted and removed daily until there were 2 consecutive days without finding a pupal cast off since the start of emergence.	
Domain 6: Confounding / 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 in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
Metric 21:	Statistical Methods	High	Analysis of variance, arcsine transformation, and the least significance test were used to analyze data.	
Metric 22:	Reporting of Data	High	Control and exposure response data were reported in Table 3 and were adequate for the outcomes of interest.	
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.	
Additional Comments:	This portion of the evaluation was on the chronic toxicity of DEHP on midge emergence. Development and growth was selected as the outcome of interest.			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2: Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent controls (ethanol) were used at 0.12 ml/L.	
	Metric 5: Negative Control Response	High	Control responses are reported 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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The chronic exposures were conducted with a flow-through system for continual renewal of the chemical. The setup and flow-rate were described well on page 32/62.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and the control.	
	Metric 9: Measurement of Test Substance Concentration	Medium	GC was used to verify the concentrations from the chronic exposure on page 25/62.	
	Metric 10: Exposure Duration and Frequency	High	The chronic exposures for midge emergence ranged from 20-40 days for hydrosol substrate and 20 - 35 days for sand substrate.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The measured concentrations are reported in tables within the results section beginning on page 35/62.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The chronic exposures are mostly below the published solubility value published in the final scope for DEHP (0.27 mg/l).	
Domain 4: Test Organism				
	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 Conditions	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 were not well described.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the chronic exposures.	
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<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972			
Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	No significant differences in emergence were observed for the chronic exposures.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The percent data was arc sin square-root transformed and Least Significant Difference tests were performed.
	Metric 22:	Reporting of Data	Medium	Emergence is reported in total numbers for each concentration, compound, and day of exposure in tables presented in the results section.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: None				
<b>Overall Quality Determination</b>			<b>Medium</b>	

<b>Study Citation:</b>	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ).		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1332972; Linked HERO ID(s): 813673, 1332972		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.
	Metric 2: Test Substance Source	Low	The source was listed as from Monsanto, but it was not analytically verified.
	Metric 3: Test Substance Purity	Low	No purity was reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Solvent controls (ethanol) were used at 0.12 ml/L.
	Metric 5: Negative Control Response	High	Control responses are reported (table 10 and 11) for the reproductive study.
	Metric 6: Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The reproductive study was conducted with a flow-through system for continual renewal of the chemical. The setup and flow-rate was described well on page 32/62.
	Metric 8: Consistency of Exposure Administration	High	The exposure administration appeared consistent among treatments and the control.
	Metric 9: Measurement of Test Substance Concentration	Medium	GC was used to verify the concentrations from the reproduction exposure on page 25/62.
	Metric 10: Exposure Duration and Frequency	High	The reproduction study was conducted with previously exposed adults from the previously chronic study.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	The measured concentrations are reported in tables within the results section beginning on page 35/62.
	Metric 12: Testing at or Below Solubility Limit	Medium	The exposures for the reproduction study were all under solubility limit except for one concentration at 0.362 mg/l with the published solubility value in the final scope for DEHP (0.27 mg/l).
Domain 4: Test Organism			
	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 Conditions	High	The pretreatment conditions were listed and similar to the chronic exposures.
	Metric 15: Number of Organisms and Replicates per Group	Low	The replication and housing groups for this work were not well described.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the chronic exposures.
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<b>Study Citation:</b> Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge ( <i>Chironomus plumosus</i> ). <b>Duration:</b> Overall Duration: > 21 days; Exposure Duration: > 21 days <b>Exposure Route, Media, Path:</b> Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) <b>Taxa, Species, Age:</b> Invertebrate; Arthropods; <i>Chironomus plumosus</i> ; Adult <b>Health Outcome:</b> Reproductive/Teratogenic <b>Chemical:</b> Di-ethylhexyl phthalate (DEHP) <b>HERO ID:</b> 1332972; Linked HERO ID(s): 813673, 1332972				
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	No significant differences in emergence were observed for the chronic exposures. From Page 34/62 - "Studies using both sand and hydrosol substrates showed DEHP had no effect on midge reproduction. ....Likewise, neither reproductive parameter was affected by DEHP concentrations up to 362 ug/l when sand was used as a substrate (Table 11)."
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothing was reported to indicate differences among treatment groups with environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The percent data was arc sin square-root transformed and Least Significant Difference tests were performed.
	Metric 22:	Reporting of Data	High	The reproductive parameters are presented in Table 10 and 11.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments: None				
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859131			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The chemical was identified by name (di(2-ethylhexyl) phthalate, DEHP) and CASRN (117-81-7).	
Metric 2:	Test Substance Source	Low	The source was identified (Sigma-Aldrich, USA), but the test substance identity was not analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A concurrent solvent control (0.01% ethanol) was used.	
Metric 5:	Negative Control Response	High	The biological response of the solvent control group was reported and adequate (represented in bar graphs by letter C of Figures 1 through 5).	
Metric 6:	Randomized Allocation	Medium	Authors reported that groups of 20 larvae were selected randomly and exposed to aqueous solutions of DEHP without sediment.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the nominal concentrations were not verified during the study.	
Metric 8:	Consistency of Exposure Administration	Low	Details of the exposure administration were not reported.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
Metric 10:	Exposure Duration and Frequency	Low	Exposure durations for the assessment of the molecular effects were hardly described in the methods. The results (Figures 1 through 5) made it more clear to understand the exposure duration because the figures show that data were collected 24, 48, 72, and 96 h post-exposure and for a group at 24 h post-exposure followed by 24 h of depuration. OECD Test No. 235: <i>Chironomus</i> sp., Acute Immobilization Test describes a range of concentrations of the test substance in water-only vessels for a period of 48 h. Given that the authors assessed molecular effects and not mobilization or mortality, the exposure duration of 24h is also appropriate.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable for the molecular effects. While the actual number of exposure groups was not reported in the methods, except for GST activity, the authors only reported that gene expression analyses were carried out at four doses: 10 <sup>−3</sup> , 10 <sup>−2</sup> , 10 <sup>−1</sup> , and 1 μg/L. GST activity was carried out at two doses: 1 and 10 <sup>3</sup> μg/L.	
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<b>Study Citation:</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859131			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	Medium	All concentrations, except for 10^3 μg/L in the GST activity assay, were below the DEHP water solubility limit (270 ug/L). The authors reported using ethanol as the solvent at 0.01%, but the authors did not offer details on whether 0.01% ethanol was sufficient to dissolve 1,000 ug/L DEHP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported. Moreover, the authors reported using the fourth instar larvae. However, OECD Test No. 235: <i>Chironomus</i> Acute Immobilization Test indicates that the first instar larvae of <i>Chironomus riparius</i> are most suitable for the acute waterborne toxicity studies with <i>Chironomus</i> because they have been shown to be the most sensitive larval stage. Further, the first instar is free swimming and therefore not stressed by the absence of sediment.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Study authors reported how cultures were maintained. There was no reason to believe that the pretreatment conditions were not the same across treatment groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize molecular effects: The authors reported using groups of 20 larvae for DEHP solutions in four independent experiments, and groups of five surviving larvae were randomly selected and used for RNA or protein extraction.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions before the exposure were described, but during exposure, they were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (semi-quantitative RT-PCR and GST activity) was clearly reported for the outcome of interest (molecular effects).
	Metric 18:	Consistency of Outcome Assessment	High	There is no evidence to suggest that the molecular effects were not assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	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 to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed and described adequately (ANOVA, followed by Games Howell's or Bonferroni's post hoc tests for normally distributed data or Kruskal-Wallis' test for not normally distributed data).
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<b>Study Citation:</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3859131

Domain	Metric	Rating	Comments
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Figures 1 through 5).
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The goal of the study was to analyze the molecular effects of DEHP exposure on <i>Chironomus riparius</i> larvae by evaluating the enzyme activity of glutathione S-transferase (GST) and transcriptional activity of genes related to crucial cell systems. The authors also conducted survival tests to examine mortality. This form was used to evaluate the molecular effects including the enzyme activity of glutathione S-transferase (GST) and transcriptional activity of genes.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859131			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name (di(2-ethylhexyl) phthalate, DEHP) and CASRN (117-81-7).
	Metric 2:	Test Substance Source	Low	The source was identified (Sigma-Aldrich, USA), but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not reported for the larval survival studies paragraph in the methods (only concentration range of 10 <sup>-3</sup> to 10 <sup>5</sup> µg/L was reported) or in the results, which were only described in the text (no tables or figures).
	Metric 5:	Negative Control Response	Uninformative	A concurrent negative control group was not reported in the methods (only concentration range of 10 <sup>-3</sup> to 10 <sup>5</sup> µg/L was reported) or in the results, which were only described in the text (no tables or figures).
	Metric 6:	Randomized Allocation	Medium	Authors reported that groups of 20 larvae were selected randomly and exposed to an aqueous solutions of DEHP without sediment.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the nominal concentrations were not verified during the study.
	Metric 8:	Consistency of Exposure Administration	Low	Details of the exposure administration were not reported. Moreover, DEHP degrades overtime, and authors did not mention replacing DEHP during the exposure duration of up to 96 h.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Uninformative	There was ambiguity in exposure durations for each of the tests, and most importantly, some exposures were only for 24 h. The authors conducted a waterborne exposure without sediment for 24h and for "longer treatments (48 to 96 h)" at the four lowest doses (10 <sup>-3</sup> to 1 µg/L) as per methods and description in the results. No other details were reported. This study was more similar to an acute waterborne toxicity test than a sediment toxicity test. Rationale for ranking: According to EPA TG OCSPP 850.1790 Chironomid Sediment Toxicity Test, Part I (the aqueous exposure test) the exposure period is 14 days. OECD Test No. 235: <i>Chironomus</i> sp., Acute Immobilization Test describes a range of concentrations of the test substance in water-only vessels for a period of 48 h.

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<b>Study Citation:</b>	Herrero, Ó., Morcillo, G., Planelló, R. (2017). Transcriptional deregulation of genetic biomarkers in <i>Chironomus riparius</i> larvae exposed to ecologically relevant concentrations of di(2-ethylhexyl) phthalate (DEHP). PLoS ONE 12(2):e0171719.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859131			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	For the larval survival study, the actual number of exposure groups was not reported. The authors only reported the range as 10 <sup>-3</sup> to 10 <sup>5</sup> µg/L, and they indicated that the four lowest doses were 10 <sup>-3</sup> to 1 µg/L. So, one can deduce that the exposure groups for the survival study were 10 <sup>-3</sup> , 10 <sup>-2</sup> , 10 <sup>-1</sup> , 1, 10 <sup>2</sup> , 10 <sup>3</sup> , 10 <sup>4</sup> , and 10 <sup>5</sup> µg/L.	
	Metric 12: Testing at or Below Solubility Limit	Low	Several concentrations (nominal concentrations ranged from 10 <sup>-3</sup> to 10 <sup>5</sup> µg/L) exceeded the DEHP water solubility limit (270 ug/L). The authors reported using ethanol as the solvent at 0.01%, but the authors did not offer details on whether 0.01% ethanol was sufficient to dissolve 100,000 ug/L DEHP.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported. Moreover, the authors reported using fourth instar larvae. However, OECD Test No. 235: <i>Chironomus</i> Acute Immobilization Test indicates that the first instar larvae of <i>Chironomus riparius</i> are most suitable for the acute waterborne toxicity studies with <i>Chironomus</i> because they have been shown to be the most sensitive larval stage. Further, the first instar is free swimming and therefore not stressed by the absence of sediment.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Study authors reported how cultures were maintained. There is no reason to believe that the pretreatment conditions were not the same across treatment groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors reported using groups of 20 larvae for DEHP solutions in four independent experiments. OECD Test No. 235: <i>Chironomus</i> Acute Immobilization Test indicates that at least 20 larvae, preferably divided into four groups of five larvae each, should be used for each test concentration and for controls. Therefore, the number of organisms per treatment group was exceeded, and the replicates were as expected.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions before the exposure were described, but during exposure, they were not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology (calculation of survival rates after 24, 48, 72, or 96 h.) was partially reported for the outcome of interest (survival/mortality rate).	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
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 to suggest differences among groups.	

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during <i>Chironomus riparius</i> development. Science of the Total Environment 470-471:1003-1011.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519014			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The DEHP was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was not reported.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the DEHP was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	There were two separate studies that were reported on in this reference. The first portion looked at the effect of temperature alone, and the second portion looked at the effect of DEHP and temperature. The control for the second portion of the study was reported to be 0.5mg/L DEHP exposure at 20C. This is not a true negative control. However, the first portion of the study had an experimental group conducted at 20C only, so it is possible that a comparison could be made. It did not appear that the two studies were run concurrently, and direct comparisons between the studies were not made in the paper.	
	Metric 5: Negative Control Response	Medium	It is possible that negative control comparisons could be made using the first portion of the study that looked at the effect of temperature alone since the second portion of the study considered a DEHP exposure of 0.5mg/L at 20C was considered the negative control. It is important to note that this is not a true negative control since there is still exposure to DEHP. If the first portion of the study is to be used for negative control results, data can be found in Fig. 2, 3, and 4.	
	Metric 6: Randomized Allocation	Low	It was not reported how the <i>C. riparius</i> larvae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	It was not reported how the DEHP concentration was prepared. Tests were conducted in 300mL crystallizing dishes and treated with 0.5mg/L DEHP. Little other details regarding the test system were provided.	
	Metric 8: Consistency of Exposure Administration	Low	All exposures were conducted in 300mL crystallizing dishes, and a 16L:8D photoperiod was used. The exposure duration was for 24h. Little other information was provided, so it was difficult to determine how consistent the exposure administration was.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the exposure concentration was measured at any point in the study.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 24h. This appeared adequate to observe a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level, as the study goal was not to observe a dose response, but to compare exposures at different temperatures.	
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<b>Study Citation:</b>	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during <i>Chironomus riparius</i> development. <i>Science of the Total Environment</i> 470-471:1003-1011.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519014			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	Low	The test concentration appeared to be above the water solubility limit for DEHP. The testing at different temperatures may have also affected the water solubility. It is unclear if a vehicle solvent was used.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The organisms were provided from adults reared in the performing laboratory. The original strain was provided by the Korea Institute of Toxicology. 4th instar individuals approximately 11-13 days old were used for this portion of the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were cultured under similar conditions to the testing conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that 13 fourth instar larvae were used in this portion of the study and that there were 3 replicates, but it is unclear how many organisms were in each replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Organisms were reported to be raised in M4 medium in 500mL crystallizing dishes. They were fed fish food at a rate of 0.5mg per larva per day. 1cm of sand was placed at the bottom of the dish. Larvae were reared at 20C with a relative humidity of 70% with a photoperiod of 16L:8D. Testing temperatures were 10, 20, and 30C. Continuous aeration was provided. Water quality parameters were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mechanistic outcomes in terms of mRNA expressions of endocrine signaling genes and antioxidant genes.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. All organisms were assessed 24h after exposure for endocrine related genes and oxidative stress related genes.
Domain 6: 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported in section 2.5 "Data analysis."

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<b>Study Citation:</b>	Park, K., Kwak, I. S. (2014). The effect of temperature gradients on endocrine signaling and antioxidant gene expression during <i>Chironomus riparius</i> development. <i>Science of the Total Environment</i> 470-471:1003-1011.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function-Oxidative stress (including redox biology)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2519014

Domain	Metric	Rating	Comments
	Metric 22: Reporting of Data	Medium	Data was provided for the exposures in Fig. 5. Negative control data for 20C without DEHP exposure could be obtained from the temperature only portion of the study. This is a separate test from the DEHP exposure portion of the study. This data is presented in Fig. 2, 3, and 4.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The control response would have to be taken from a different portion of the study, which is a limitation when it comes to unexpected outcome. Study authors did provide measures of variability within each test.

**Additional Comments:** This evaluation was on the effect of DEHP as well as varying temperature on *C. riparius* 4th instar larvae oxidative stress and endocrine gene expression. There were two portions to this study. One portion involved an assessment of varying temperatures on *C. riparius*. The other portion involved an assessment of the effect of DEHP and varying temperature on *C. riparius*. These appeared to be two different studies that may not have been run concurrently. The DEHP portion of the study did not have a true concurrent negative control. For this portion of the study, study authors reported the negative control was 0.5mg/L DEHP at 20C. However, the temperature only portion of the study did have test group at 20C without DEHP. It is possible that this could be used as a comparator in the study with DEHP. The two different studies were not compared directly in the paper by study authors.

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in <i>Chironomus riparius</i> exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681990			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using a concurrent negative control.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	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 and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and the spacing of exposure levels. This may have contributed to the poor dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	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.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was unclear if the number of replicates was equal between treatments or if the number of organisms was similar.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	
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<b>Study Citation:</b>	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in <i>Chironomus riparius</i> exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	681990

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
Domain 6: Confounding / 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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Minor uncertainties were identified in how the study characterized unexpected outcomes.

Additional Comments: The authors characterized all outcomes as reproductive effects. This form accounts for the emergence endpoint.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in <i>Chironomus riparius</i> exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681990			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using a concurrent negative control.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	High	Exposures were administered consistently across study groups.	
	Metric 9: Administration 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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and the spacing of exposure levels. This may have contributed to a poor dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	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.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was unclear if the number of replicates was equal between treatments or if the number of organisms was similar.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest.	
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<b>Study Citation:</b>	Kim, E. J., Lee, S. K. (2004). Reduced viability of F1 egg ropes in <i>Chironomus riparius</i> exposed to di-2-ethylhexyl phthalate (DEHP). Journal of Environmental Biology 25(3):259-261.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	681990

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 / 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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Minor uncertainties were identified in how the study characterized unexpected outcomes.

Additional Comments: The authors characterized all outcomes as reproductive effects.

## Overall Quality Determination

## Medium



<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681634			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].	
Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).	
Metric 3:	Test Substance Purity	High	The purity was reported as 99%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.	
Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.	
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured; only nominal concentrations were reported.	
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, <1= 0.2%) was used to aid in dissolution of the test compound.	
Domain 4: Test Organism				
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.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects (20 larvae per test vessel, 9 replicates per test concentration).	
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<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	681634		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reasonably described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.
Additional Comments: This study utilized a developmental based study duration.			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681634			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured; only nominal concentrations were reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, </= 0.2%) was used to aid in dissolution of the test compound.
Domain 4: Test Organism				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects (20 larvae per test vessel, 9 replicates per test concentration).
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681634			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18:	Consistency of Outcome 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reasonably described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.
Additional Comments:	This study utilized a developmental based study duration. Growth and development time were evaluated in this form.			
Overall Quality Determination		High		

<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681634			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory; the manufacturer was identified (Junsei Chemical Co. Ltd., Japan).
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups – a solvent control group and a non-solvent control group.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured; only nominal concentrations were reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. In addition, a solvent (analytical grade acetone, </= 0.2%) was used to aid in dissolution of the test compound.
Domain 4: Test Organism				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects (20 larvae per test vessel, 9 replicates per test concentration).
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Kwak, I. S., Lee, W. (2005). Endpoint for DEHP exposure assessment in <i>Chironomus riparius</i> . Bulletin of Environmental Contamination and Toxicology 74(6):1179-1185.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus riparius</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681634			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18:	Consistency of Outcome 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reasonably described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes.
Additional Comments:	This study utilized a developmental based study duration. This form addresses the sex ratio outcome.			
Overall Quality Determination		High		

<b>Study Citation:</b>	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in <i>Chironomus tentans</i> (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. <i>Chemosphere</i> 65(6):1074-1081.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	492760			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Medium	The test chemical was identified by name.	
	Metric 2: Test Substance Source	High	The source was stated as Fluka.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was unclear if authors used a solvent control, although, use of a control was reported and shown in Figure 5.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate and shown in Figure 5.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental set-up was explained; the addition of the chemical to the beakers had few details.	
	Metric 8: Consistency of Exposure Administration	Low	Few details of the exposure administration were reported. There was no mention of how the controls were prepared.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	This was a 48 hour exposure for assessing body weight.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	Three exposure concentrations and a control were utilized in this study. The DEHP concentrations cover two orders of magnitude. Additional doses could have been added to the low end for a better dose response representation.	
	Metric 12: Testing at or Below Solubility Limit	Low	The exposure concentrations stated in Figure 5 and in the text for body weight are above the solubility limits for DEHP in water (5 mg/L, 50 mg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the test organisms was stated.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The test organisms were not acclimatized to the test beakers prior to chemical exposure.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 10 test organisms per beaker. The number of replicate beakers (if any) per chemical treatment was not stated.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in <i>Chironomus tentans</i> (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. <i>Chemosphere</i> 65(6):1074-1081.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	492760			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	The DO and pH of the test water were not stated. Other conditions were explained satisfactorily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported how fresh weights and dry weights were obtained.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome methodology was conducted at 48 hr after the start of the exposure.
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 to suggest difference in animal attrition among treatments.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The authors utilized the parametric t test to determine significant differences.
	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	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes. A trend was reported but had high variability.
Additional Comments:	Caution may be warranted for the units of DEHP in this study as inconsistency in figures and text between doses (units) are stated for mechanistic endpoints vs weight endpoints (with supposedly the same doses utilized for both endpoints, i.e. the 1/10, 1/100, and 1/1000th of the 24 hr LC50).			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in <i>Chironomus tentans</i> (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. Chemosphere 65(6):1074-1081.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	492760			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Medium	The test chemical was identified by name.	
	Metric 2: Test Substance Source	High	The test substance was obtained from Fluka.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Use of a solvent control was not explicitly stated; a 0 mg/L treatment was included in Figure 2.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental set-up was explained; the addition of the chemical to the beakers had few reported details.	
	Metric 8: Consistency of Exposure Administration	Low	Few details of the exposure administration were reported. There was no mention of how controls were prepared.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	There was a 24 hr exposure period for the gene expression endpoint.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations and a control were utilized in this study. The DEHP concentrations cover two orders of magnitude. Additional doses could have been added to the low end for a better dose response representation.	
	Metric 12: Testing at or Below Solubility Limit	Low	It was unclear whether doses exceeded solubility in the gene expression analysis as the units in Figure 2 state both mg/L (which exceed approximate solubility of 0.3 mg/L) and ug/L for DEHP were used. The text states ug/L for gene expression results.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of <i>C. tentans</i> was stated.	
	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	There were 10 test organisms per beaker. The number of replicate beakers (if any) per chemical treatment was not stated. It is unclear if the experiment was performed thrice.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	The DO and pH of water were not stated. Other conditions were explained satisfactorily.	
	Metric 17: Outcome Assessment Methodology	High	The outcome methodology for gene expression analysis was well described.	
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<b>Study Citation:</b>	Lee, S. M., Lee, S. B., Park, C. H., Choi, J. (2006). Expression of heat shock protein and hemoglobin genes in <i>Chironomus tentans</i> (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring. <i>Chemosphere</i> 65(6):1074-1081.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	492760			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment methodology was carried out at 24 hr for all exposure groups.	
Domain 6: Confounding / 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 to suggest differences in animal attrition among the different groups.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The authors utilized parametric t test to determine significant differences.	
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group in Figure 2. There was inconsistent use of units for DEHP in Figure 2 and in the text (0.5, 5, 50 ug/L vs 0.5, 5, 50 mg/L).	
	Metric 23: Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes (e.g. high variation in group receiving the highest dose of DEHP).	
Additional Comments:	The doses utilized for DEHP are inconsistent in units (unclear which units are correct). Caution may be warranted for the units of DEHP in this study as inconsistency in figures and text between doses (units) are stated for mechanistic endpoints vs weight endpoints (with supposedly the same doses utilized for both endpoints, i.e. the 1/10, 1/100, and 1/1000th of the 24 hr LC50).			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to Chironomus tentans.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335360			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only. A CASRN number is present on the cover sheet and written on a page, but it does not appear to be original to when the document was formed.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	All solvent control animals were immobilized at 48hr (page 7/10). The treatment concentration (10 mg/L DEHP) and control were both 0% immobilized.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	Not enough information was reported to adequately assess this metric.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only one concentration was used with no effects reported.	
	Metric 12: Testing at or Below Solubility Limit	Low	The test concentration was 10mg/L, which is over the solubility reported in the final Scope for DEHP (0.2 mg/L).	
Domain 4: Test Organism				
	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.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to <i>Chironomus tentans</i> .			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335360			
Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment 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.
	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	N/A	Only one concentration was assessed.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None				

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to Chironomus tentans.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335360			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1: Test Substance Identity	Low	Chemical identified by name only. CASN number is present on cover sheet and written on page, but do not appear to be original to when document was formed.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent and solvent negative control groups.	
	Metric 5: Negative Control Response	High	All solvent control animals were immobilized at 48hr (page 7/10). The treatment concentration (10 mg/L DEHP) and control were both 0% immobilized.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	Not enough information reported to adequately assess this metric.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	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 one concentration used with no effects reported.	
	Metric 12: Testing at or Below Solubility Limit	Low	The test concentration was 10mg/L which is over the solubility reported in the final Scope for DEHP (0.2 mg/L).	
Domain 4: Test Organism	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.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to maintenance of health.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
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<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di (2-ethylhexyl) phthalate to <i>Chironomus tentans</i> .			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335360			
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 / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Only one concentration assessed.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This form is for the mortality outcome reported in the paper. Test concentrations and corresponding percent mortality data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50 and 95% confidence intervals.			

**Overall Quality Determination****Medium**

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

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Although adequate conditions were described prior to testing, details are lacking for the acute toxicity test solution.
	Metric 17: Outcome Assessment Methodology	Medium	Mortality was the endpoint of interest, but there were no details on how this was determined in <i>C. tentans</i> (e.g. not moving for a certain period of time).
	Metric 18: Consistency of Outcome Assessment	High	Mortality was determined at 24 hr of exposure.
Domain 6: 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 or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences among study groups unrelated to exposures.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The study authors used probit analysis with confidence intervals to determine effect concentrations (EC10, EC50 and EC90).
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented in Table 1, but control outcomes were not provided for comparison.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Confidence intervals for LC10, LC50, and LC90 values were presented in Table 1 (unclear how many replicate experiments were conducted).

Additional Comments: The mortality endpoint in *C. tentans* received a low rating due to lack of experimental details and limited presentation of the data.

## Overall Quality Determination

**Low**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679311			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The chemical was identified as a by name. Further details such as CASRN were provided in Call et al 2001.	
Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).	
Metric 3:	Test Substance Purity	High	The purity was identified as 99% in the cited reference Call et al 2001.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.	
Metric 5:	Negative Control Response	High	The biological response of control group was appropriate as shown in Table 5.	
Metric 6:	Randomized Allocation	Low	Random allocation was not stated.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and addition of sediment to the test beakers were described in detail.	
Metric 8:	Consistency of Exposure Administration	High	The exposure consistency was reported and consistent.	
Metric 9:	Measurement of Test Substance Concentration	High	The concentrations were measured using HPLC as described in the methods, and they were referenced in Call et al 2001.	
Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design and followed cited methods (EPA, 1994).	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).	
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation of the test organisms prior to exposure was not reported.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.	

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	679311		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity on days 1 and 9.
	Metric 17: Outcome Assessment Methodology	High	The sediment was sieved, and survivors were collected, dried, and weighed.
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There was no reported differences among study groups that could influence outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were 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	High	Survival data from toxicity tests were summarized using the trimmed Spearman-Kärber method. Dry weight data were analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStat Program.
	Metric 22: Reporting of Data	High	Treatment and control data were reported in Table 5. Results were represented as the average dry weight per individual.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679311			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. Further details such as CASRN were provided in Call et al 2001.
	Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	The purity was identified as 99% in the cited reference Call et al 2001.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	The biological response of the control group was appropriate as shown in Table 5.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and the addition of sediment to test beakers were described in detail.
	Metric 8:	Consistency of Exposure	High	Exposure consistency was reported and consistent.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods, and they were referenced in Call et al 2001.
	Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed cited methods (EPA, 1994).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation of the test organisms prior to exposure was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679311			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity on days 1 and 9.	
	Metric 17: Outcome Assessment Methodology	Medium	Survivor count was determined after the 10 day exposure, but it was not reported as percent mortality.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups that could influence outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were 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	High	Survival data from toxicity tests were summarized using the trimmed Spearman-Kärber method. Dry weight data were analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStat Program.	
	Metric 22: Reporting of Data	Medium	Data were reported in Table 5, however, results were pooled among replicates.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.	
Additional Comments: None				
Overall Quality Determination		High		

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Chironomus tentans</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	The environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.	
	Metric 18: Consistency of Outcome Assessment	High	The 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	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.	
Additional Comments:	None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Test substance nomenclature was reported without a CASRN.	
	Metric 2: Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.	
	Metric 3: Test Substance Purity	High	At least 95% purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was reported.	
	Metric 5: Negative Control Response	High	The control response was acceptable.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or the measures taken to prevent volatilization were not reported.	
	Metric 8: Consistency of Exposure Administration	High	The exposure administration was consistent across groups.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.	
	Metric 10: Exposure Duration and Frequency	High	The duration and the frequency of the exposure were appropriate for the test.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.	
	Metric 12: Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Immobilization
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

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 Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**



<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to <i>Daphnia magna</i> (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316223			
Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The chemical is identified by name and CASRN. No other verification is provided.
	Metric 2:	Test Substance Source	Low	Chemicals were provided by the General Electric Company in 1-L amber glass bottles. No analytical verification or additional information was provided.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure).
	Metric 5:	Negative Control Response	High	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 Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	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 test concentrations were very low at the end of the test, but the authors reported all of the concentrations. Any endpoint should be calculated in terms of the mean-measured concentration rather than the initial measured concentration, which is reported in the study.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods. Analytical technologies used were highly sensitive (GC-MS).
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (acute <i>Daphnia</i> study of 48-hour duration).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Test concentrations were spaced with the intention of bracketing the limit of solubility, but the actual measured test concentrations were very low. The results of this test can only be used to characterize the toxicity up to the limit of solubility of the test medium, which may or may not reflect the solubility reported in other sources.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The authors reported that the initial test featured a film that captured and killed a significant number of the daphnia. A solvent should have been used. As this did not cause excess mortality in the test, this was not determined to negatively affect the results of the test.
Domain 4: Test Organism				

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<b>Study Citation:</b>	Bionomics., Springborn (1984). Acute toxicity of fourteen phthalate esters to <i>Daphnia magna</i> (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316223			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	The source (and sex if relevant) of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups. As excess mortality was not observed in the controls, this was not determined to adversely affect the outcome.
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 daphnia/replicate is far lower than the guideline required 20 daphnia/replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and the biomass loading was appropriate.
	Metric 17:	Outcome Assessment Methodology	Low	Significant deficiencies in the reported outcome assessment methodology were identified. The test was intended to quantify the mortality up to the limit of solubility of the chemical. This was not accomplished due to the extremely low solubility in the test medium. In both the definitive and the corroborative tests, the authors report that a film of insoluble test material formed on the surface that entrapped some of the test organisms but didn't result in mortality in the corroborative test.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable 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	Low	A film of insoluble test material may have affected the test organisms.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	No mortality was observed, so statistical analysis was not needed.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not conduct a solubility test in the test medium that would have explained why the measured test concentrations were so low in comparison to the reported solubility of the test material elsewhere.
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<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of fourteen phthalate esters to <i>Daphnia magna</i> (final report).
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1316223

Domain	Metric	Rating	Comments
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 apparently also formed a film, but no mortality was observed. Results should be calculated in terms of mean-measured test concentration of the concentrations at the beginning and end of the tests, rather than only at the initiation of the test, which is how the authors report the study results. Furthermore, no solubility test was conducted with the test medium, so the results may not be representative of the solubility of DEHP in a natural aquatic system.		

**Overall Quality Determination****Medium**

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

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

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. <i>Chemosphere</i> 11(4):417-426.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Immobilization
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334281

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Though statistical analysis was not conducted, there was no effect seen at any concentration.
	Metric 22: Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Huang, B., Li, D., Yang, Y. (2016). Joint toxicity of two phthalates with waterborne copper to <i>Daphnia magna</i> and <i>Photobacterium phosphoreum</i> . Bulletin of Environmental Contamination and Toxicology 97(3):380-386.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5750702			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported, but the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Medium	It was mentioned that the chemicals used were analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test media preparation methods were reported but did not provide the measures taken to minimize loss of test substance before and during the exposure. The concentration of the test substance was measured during the study.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using HPLC. The percent recovery of spiked test substance was within an acceptable range. Measured concentrations were similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	Medium	Minor limitations in exposure frequency and duration of exposure were identified -acute daphnid toxicity study of 24-hour duration as opposed to 48 hours.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	7 exposure groups and a control were tested.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate. No effects on biological responses were observed in the solvent control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 10 daphnids per test vessel, and they were tested in triplicate.
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<b>Study Citation:</b>	Huang, B., Li, D., Yang, Y. (2016). Joint toxicity of two phthalates with waterborne copper to <i>Daphnia magna</i> and <i>Photobacterium phosphoreum</i> . Bulletin of Environmental Contamination and Toxicology 97(3):380-386.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5750702		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	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 Standard Method of China (GB/T 13266-1991).
	Metric 17: Outcome Assessment Methodology	Low	The outcome (immobilization of daphnids) assessment methodology was not clearly reported.
	Metric 18: Consistency of Outcome Assessment	High	Immobilization was recorded after 24 hours of exposure in all treatment groups and the control.
Domain 6: Confounding / Variable 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 on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately. The methods for calculating effect ratio ( y-axis of Fig 1) and EC 50 values were not provided.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group via figures. The methods for calculating effect ratio ( y-axis of Fig 1) were not provided. EC 50 values were given without confidence intervals in the text .
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: Data analysis methods were not provided. The EC 50 value was given without confidence intervals.			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Larvae			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789536			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The test substance was identified definitively. Nomenclature, CASRN, and structure were reported.	
Metric 2:	Test Substance Source	High	DEHP was purchased from Merck Eurolab (Stockholm, Sweden), but the test substance identity was NOT analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	High	Percent purity was reported as >98%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
Metric 5:	Negative Control Response	Low	The biological response of the control group was not clearly reported. It was stated that tests were repeated if the mortality in the control group exceeded 5%.	
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
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 exposure.	
Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were measured but not reported.	
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	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 Organism				
Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per concentration with 5 animals used in each replicate.	

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Larvae		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	789536		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate 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	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 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 Presentation and Analysis			
Metric 21:	Statistical Methods	High	EC10 and EC 50 values were calculated using probit analysis with maximum likelihood estimation.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained. Authors noted surface entrapment of test animals in the highest tested concentration, but these were excluded from the calculation of EC 10 and EC 50 values.
Additional Comments:	The exposure concentrations, spacing of exposure levels, and control response were not reported. Measured concentrations were not reported. Mortality data were not provided for each of the treatment groups and control. Only 24 and 48 hour EC 10 and EC 50 values were reported.		
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070913			
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	High	"Optimized LC and MS/MS parameters for each analyte were based on reported information and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Reporting omissions may have an impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The high concentration was measured as fresh and aged but not as the actual experimental doses.	
	Metric 10: Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 (10) were reported and sufficient to characterize toxicological effect.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M., A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3070913

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
Domain 6: Confounding / 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 were no differences among groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Some of the statistical analysis methods were described in Section 2.7 (page 5/10), but methods on EC estimation methods were not reported.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The behavioral outcome is to account for feeding inhibition.

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070913			
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	High	"Optimized LC and MS/MS parameters for each analyte were based on reported information and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Reporting omissions may have an impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The high concentration was measured as fresh and aged but not as actual experimental doses.	
	Metric 10: Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 (10) were reported and sufficient to characterize a toxicological effect.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	The environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.	
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<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3070913

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 / 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 were no differences among groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was assumed to be performed, but it was not described in the statistical analysis section (2.7 page 5/10).
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation was for body length from Table S3.

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070913			
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	High	”Optimized LC and MS/MS parameters for each analyte were based on reported information and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves.”	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control groups was not reported for molts.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Reporting omissions may have an impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The high concentration was measured as fresh and aged but not as the actual experimental doses.	
	Metric 10: Exposure Duration and Frequency	Medium	This metric was marked medium based on uncertainties in exposure duration which was based on developmental stage.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number or range of concentrations was not reported.	
	Metric 12: Testing at or Below Solubility Limit	Low	Reporting omissions prevented the determination of whether exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 (10) were reported and sufficient to characterize a toxicological effect.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.	

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<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M., A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3070913

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment 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.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical estimation methods of the molt LOEC concentration were not reported.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation was for molt.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070913			
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	High	”Optimized LC and MS/MS parameters for each analyte were based on reported information and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves”
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported for mortality.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Reporting omissions may have an impact on results.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The high concentration equivalent was measured as fresh and aged but not as actual experimental doses.
	Metric 10:	Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration, which was based on developmental stage.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number or range of concentrations was not reported.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	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	Medium	The numbers of test organisms and replicates (10) were reported and sufficient to characterize toxicological effect.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.

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<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V., M., A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3070913

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	These are minor uncertainties that are unlikely to have a substantial impact on results.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment 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.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	No statistical analysis on mortality was reported.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation is for the mortality outcome- From Section 3.2, "None of the studied compounds affected survival at the tested concentrations."

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3070913			
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	High	"Optimized LC and MS/MS parameters for each analyte were based on reported information and are depicted in Table S1. Quantification was based on external calibration standard of 8 point curves."	
	Metric 3: Test Substance Purity	Low	Purity or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Reporting omissions may have an impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The high concentration was measured as fresh and aged but not as the actual experimental doses.	
	Metric 10: Exposure Duration and Frequency	Medium	This metric was graded as medium based on uncertainties in exposure duration which was based on development stage.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 (10) were reported and sufficient to characterize toxicological effect.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of the test system were not well reported. It was not clear if they were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Medium	There are minor uncertainties that are unlikely to have a substantial impact on results.	
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<b>Study Citation:</b>	Jordão, R., Garreta, E., Campos, B., Lemos, M. F., Soares, V.,M, A.M., Tauler, R., Barata, C. (2015). Compounds altering fat storage in <i>Daphnia magna</i> . Science of the Total Environment 545-546(Elsevier):127-136.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; clone F; Juvenile
<b>Health Outcome:</b>	Nutritional & Metabolic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3070913

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 / 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 were no differences among groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Some of the statistical analysis methods were described in Section 2.7 (page 5/10), but methods on EC estimation were not reported.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: Nile red fluorescence was used to determine lipid content. Data/results were presented in Table 2, Page 6/10.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> .			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335345			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by name. No CASRN or structure were reported.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control and the solvent control were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	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 and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Low	All test concentrations have exceeded solubility, but a solvent was used to aid solubility. The lowest concentration in the bioassay was 1 mg/L, and the solubility listed in the Final Scope for DEHP is 0.27 mg/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.

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<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> .			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335345			
Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18:	Consistency of Outcome 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	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None				

**Overall Quality Determination****High**

<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> in the presence of fulvic acid.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335353			
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 was obtained from an individual, and the identity was not analytically verified by the performing laboratory."DEHP (Lot-#QL-1000), a clear liquid, was obtained from Robert H. Mills (MIC) and was used as the test chemical."	
	Metric 3: Test Substance Purity	Low	The purity or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group "consisting of the same dilution water and conditions but with no test compound or ethanol," as well as a positive control "consisting of the same dilution water, conditions, and ethanol."	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit; ethanol was used to aid solubility.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.	

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<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di-2-ethylhexyl phthalate (DEHP) to <i>Daphnia magna</i> in the presence of fulvic acid.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1335353		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome 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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	Muller (1983). Determination of the acute toxicity of di-2-ethylhexyl-phthlat (dehp) to the waterflea daphnia magna straus.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	11328251		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Breeding conditions prior to the test were reported and appropriate. Test conditions were reported and appropriate. Water chemistry measurements for the test are shown in Tables 1, 2, and 3.
	Metric 17: Outcome Assessment Methodology	High	Daphnia magna were assessed at zero, 24, and 48 hours for inability to swim after gentle agitation of the test vessels. These data were used to determine the EC values.
	Metric 18: Consistency of Outcome Assessment	High	The assessment of Daphnia magna mobility at the desired timepoints was done consistently across all test groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Temperature, pH, and oxygen concentrations were reported across all test concentrations.
	Metric 20: Outcomes Unrelated to Exposure	High	There was no information to suggest differences in test organisms among concentration groups that could influence the outcome assessment. There was no reason to suggest anything other than exposure concentrations were different among experimental units.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were not described but EC values were calculated in the results. A 95% confidence interval was shown in the results section, but no value was indicated for it (blank lines next to it). These results are shown on page 8 of the pdf. Standard LC50 calculations were performed. Very little immobilization was observed across treatments. Thus, LC50s and CIs could not be calculated for "no effect."
	Metric 22: Reporting of Data	High	Data for Daphnia magna able to swim after 0, 24, and 48 hours was shown in Table 4 for all test concentrations (averaged over all replicates). Table 1A shows data for Daphnia magna able to swim for all replicates, across all investigated timepoints and test concentrations.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes. DEHP did not affect daphnids.
Additional Comments: This evaluation is for the assessment of immobilization in Daphnia magna neonates exposed to DEHP for 48 hours.			
<b>Overall Quality Determination</b>		<b>High</b>	

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

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Although adequate conditions were described prior to testing, details are lacking for the acute toxicity test solution.
	Metric 17: Outcome Assessment Methodology	Medium	Swimming inhibition was utilized as an endpoint. More detail could have been provided of what authors determined as inhibition.
	Metric 18: Consistency of Outcome Assessment	High	Swimming inhibition was determined after 24 hr of exposure.
Domain 6: 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 or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences among study groups unrelated to exposures.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The study authors used probit analysis with confidence intervals to determine effect concentrations (EC10, EC50 and EC90).
	Metric 22: Reporting of Data	Low	Effective concentrations for exposure-related findings were presented in Table 1, but control outcomes were not provided for comparison.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Confidence intervals for EC10, EC50, and EC90 values were presented in Table 1 (unclear how many replicate experiments were conducted).

Additional Comments: The immobilization endpoint in *D. magna* received a low rating due to lack of experimental details and limited presentation of the data.

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator <i>Daphnia magna</i> indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. <i>Environmental Science &amp; Technology</i> 49(12):7400-7410.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966135			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance	Metric 1:	Test Substance Identity	Medium	The correct nomenclature and figure of the structure were reported. The compound was referred to as "bis(2-ethylhexyl) phthalate." This is a synonym for DEHP.
	Metric 2:	Test Substance Source	Low	The source was listed as Aldrich with no analytical verification reported.
	Metric 3:	Test Substance Purity	Low	No purity or grade were reported.
Domain 2: Test Design	Metric 4:	Negative Controls	Low	The actual concentration of DMSO in the solvent control was not reported, but it was reported to be the same as the one used in the exposure concentration, which was reported to be 1/10 of the LC50 value.
	Metric 5:	Negative Control Response	Low	The control values for the gene expression microarray were averaged and then used as reference values to create fold changes in specific genes in addition to authors use of housekeeping genes (actin and GAPDH) to normalize responses. However, the response of just the control was not reported.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Characterization	Metric 7:	Experimental System/Test Media Preparation	Low	The study authors did not say explicitly what was done (e.g., flow through or static exposure). Exposure concentrations also were not measured. The material used for the exposures (e.g., glass, plastic) was not mentioned. Sorption out of the exposure water may have occurred.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently among treatments and controls.
	Metric 9:	Measurement of Test Substance Concentration	Low	Concentrations were reported as nominal (1/10 of LC50), and no analytical verification was performed.
	Metric 10:	Exposure Duration and Frequency	High	The extraction of genes for a microarray could capture regulation of genes from an initial 48 hr exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The micro array was conducted by exposing 15-20 adult Daphnids for 48 hour to a single treatment concentration (1/10th LC50).
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentration was 1/10th the LC50. Authors indicated that 3.3 mg/L was the LC50 so 0.3 mg/L was the exposure concentration. The solubility from the Final Scope is listed at 0.27 mg/L.
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<b>Study Citation:</b>	Scanlan, L. D., Loguinov, A. V., Teng, Q., Antczak, P., Dailey, K. P., Nowinski, D. T., Kornbluh, J., Lin, X. X., Lachenauer, E., Arai, A., Douglas, N. K., Falciani, F., Stapleton, H. M., Vulpe, C. D. (2015). Gene transcription, metabolite and lipid profiling in eco-indicator <i>Daphnia magna</i> indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. <i>Environmental Science &amp; Technology</i> 49(12):7400-7410.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2966135			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The <i>Daphnia</i> were cultured asexually in a growth chamber and were acquired from ARO in New Hampshire. 14-d old daphnids were exposed in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	The <i>Daphnia</i> were maintained in COMBO media, and initial pH was reported. pH during the bioassay was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It does not appear that the microarray portion of this study was replicated. The micro array was conducted by exposing 15-20 adult <i>Daphnids</i> for 48 hour to a single treatment concentration (1/10th LC50).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The culture media formula is listed in Table S1. Feeding protocols and water pH are described in the methods section.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods addressed the outcome of interest (gene expression compared to control conditions) and were sensitive enough to record data.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently applied across the one exposure and control treatment groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among treatments and control to indicate that factors outside of the compound influenced the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	The authors did not present information to suggest that animal health interfered with the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The analysis of the microarray was presented on page 3/11 in the "Gene ontology, pathway enrichment and cluster analysis" section.	
	Metric 22: Reporting of Data	High	Figure 4 and table 1 (page 5/11) present the results of the microarray.	
	Metric 23: Explanation of Unexpected Outcomes	High	Authors indicated no unexpected outcomes.	
Additional Comments: None				
Overall Quality Determination		High		

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

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

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

Domain	Metric	Rating	Comments
<b>Overall Quality Determination</b>		<b>Medium</b>	

<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	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.	
	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	Reporting omissions are unlikely to have a substantial impact on results.	
	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	Low	Only two exposure groups were reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.	
Domain 5: Outcome Assessment				
	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.	
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5043468

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This was a 24 hr exposure that was measured at 96 hr.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	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.	
	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	Reporting omissions are unlikely to have a substantial impact on results.	
	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	Low	Only one exposure group was reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5043468

Domain	Metric	Rating	Comments
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcome.

Additional Comments: This evaluation was for the progeny assessment.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as >99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.	
	Metric 6: Randomized Allocation	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	Reporting omissions are unlikely to have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: 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/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 Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
	Metric 22:	Reporting of Data	Uninformative	Data presentation was inadequate.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This was for organisms with ephippia.				

**Overall Quality Determination****Uninformative**



<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	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.	
	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	Reporting omissions are unlikely to have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/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 Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Nutritional & Metabolic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5043468

Domain	Metric	Rating	Comments
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This portion of the evaluation was for lipid accumulation.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	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	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 Administration	Medium	Reporting omissions are unlikely to have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure (48 hr) was reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/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 Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of exposure groups and the spacing of exposure levels were suitable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
	Metric 22:	Reporting of Data	Uninformative	Data presentation was inadequate.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None				

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5498837		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).
Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).
Metric 5:	Negative Control Response	High	No control mortality was observed in the control group treated with deionized water or acetone during the 48-h exposure.
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	High	Details of exposure administration were reported, and exposures were administered consistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and <i>Daphnia magna</i> juveniles were placed in a 100-mL glass beaker containing 100 mL of test solution.
Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured and were similar to nominal concentrations, except for nominal concentration 0.06 mg/L for which the authors reported 0.00581 mg/L (given that the rest of the measured concentrations were very similar to the nominal concentrations, it is unclear if the reported value of 0.00581 mg/L was a typo with an extra 0). Moreover, there are no details of when the measured concentrations were taken, and it appears that they were not taken repeatedly such as at the beginning, throughout, and at the end of the study.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (acute toxicity in <i>Daphnia magna</i> ).
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Juveniles (< 24 h): The number of exposure groups and spacing of exposure levels were suitable for a dose response: 0.4, 0.6, 0.8, 1.0, and 1.2 mg/ L and a blank control and a solvent control (0.004% acetone, v/v).
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<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5498837			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in <i>Daphnia magna</i> .
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species ( <i>Daphnia magna</i> ) and age newly hatched juveniles (< 24 h)] for the study type. Moreover, the authors reported following test guidelines of the OECD Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. Five replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each replicate, ten <i>Daphnia magna</i> juveniles were placed in a 100-mL glass beaker containing 100 mL of test solution, and a total of 50 organisms were exposed to each concentration in the DEHP gradient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The authors reported following test guidelines of the OECD (2004), which is Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test. In addition, the authors reported that the exposures were static, nonrenewal tests and that the test animals were not fed. However, the authors did not describe specific environmental conditions such as water quality control parameters taken during the test.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (organisms were considered immobile if they would not swim within 15 s after gentle agitation) was reported and adequate for the intended outcome of interest (immobilization to determine acute toxicity, LC50).
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment protocol was reported, and there was no evidence that it was not assessed consistently across study groups.
Domain 6: Confounding / 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 were no reported differences among the study groups in environmental conditions.
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<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5498837		
Domain	Metric	Rating	Comments
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described. The lethal concentrations such as LC50 and the 95% confidence limits were calculated by Probit analysis using SPSS 21.
	Metric 22: Reporting of Data	Medium	The authors did not report the raw or mean immobilization results for each treatment group. They instead reported LC50 values at 24 and 48 hours, and they reported the effect/correlation of DEHP concentration and exposure duration on acute toxicity.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipidperoxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in <i>Daphnia magna</i> newly hatched juveniles (< 24 h) and adults (> 96 h). This form was used to evaluate the acute toxicity (immobilization) in newly hatched juveniles (< 24 h).		

**Overall Quality Determination****High**

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

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species ( <i>Daphnia magna</i> ) and age (adults (> 96 h)) for the study type (subacute antioxidant effects of DEHP). Moreover, <i>Daphnia magna</i> is an adequate species used in toxicity tests and approved by OECD and EPA.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there was no evidence that they were not the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize subacute toxicological effects. 4 replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each replicate, a total of 500 adults were placed in 5-L glass beaker. At 24 and 48 h, 250 living organisms were collected from each beaker as a replicate and divided into two samples. 100 individuals were used to measure MDA content and enzyme activity, and 150 individuals were used for RT-PCR analysis.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The authors reported that for the acute toxicity studies they followed test guidelines of the OECD (2004), which is Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test. However, the authors did not offer details on environmental conditions for the subacute experiments.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (lipid peroxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity, and catalase and glutathione-S-transferase mRNA levels) was reported and adequate for the intended outcome of interest (effects of DEHP on antioxidant responses).
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was reported and there was no evidence that it was not assessed consistently across study groups.
Domain 6: 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 were no reported differences among the study groups in environmental conditions.
Domain 7: Data Presentation and Analysis			

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

**Overall Quality Determination****High**

<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5498837		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).
Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).
Metric 5:	Negative Control Response	High	The biological responses (oxidative stress and antioxidant gene expression) were reported and adequate for the control group.
Metric 6:	Randomized Allocation	Medium	Authors reported that for the subacute exposures to examine antioxidant activity and gene expression analyses, organisms were randomly assigned to experimental units (5 L beakers).
Domain 3: Exposure 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	High	Details of exposure administration were reported and exposures were administered consistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and <i>Daphnia magna</i> juveniles were placed in a 5L glass beaker containing 5L of test solution.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (antioxidant activity and gene expression in <i>Daphnia magna</i> ).
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Juveniles (< 24 h): The number of exposure groups and the spacing of exposure levels were suitable for the antioxidant activity and gene expression assessment: 0.06 and 0.1 mg/L and a blank control and a solvent control (0.004% acetone, v/v).
Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in <i>Daphnia magna</i> .
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<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5498837

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species ( <i>Daphnia magna</i> ) and age (adults (< 24 h)) for the study type (subacute antioxidant effects of DEHP). Moreover, <i>Daphnia magna</i> is an adequate species used in toxicity tests and approved by OECD and EPA.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize subacute toxicological effects. 4 replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each replicate, a total of 600 juveniles were placed in 5-L glass beaker. At 24 and 48 h, 250 living organisms were collected from each beaker as a replicate and divided into two samples. 100 individuals were used to measure MDA content and enzyme activity, and 150 individuals were used for RT-PCR analysis.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The authors reported that for the acute toxicity studies they followed test guidelines of the OECD (2004), which is Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test. However, the authors did not offer detail on environmental conditions for the subacute experiments.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (lipid peroxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity, and catalase and glutathione-S-transferase mRNA levels) was reported and adequate for the intended outcome of interest (effects of DEHP on antioxidant responses).
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was reported, and there is no evidence that it was not assessed consistently across study groups.
Domain 6: 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	High	There were no reported differences among the study groups in environmental conditions.

Domain 7: Data Presentation and Analysis

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

**Overall Quality Determination****High**

<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult
<b>Health Outcome:</b>	Immobilization
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5498837

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The chemical was identified by name (Di-2-ethylhexyl phthalate) and CASRN (117-81-7).
Metric 2:	Test Substance Source	Low	The test substance source was identified (from SinopharmChemical Reagent Co. Ltd, Shanghai, China), but the test substance was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Medium	There was no quantified purity provided, just a description that said "All chemicals used were of analytical grade".
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported using an appropriate blank control and a solvent control (0.004% acetone, v/v).
Metric 5:	Negative Control Response	High	No control mortality was observed in the control group treated with deionized water or acetone during the 48-h exposure.
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	High	Details of exposure administration were reported, and exposures were administered consistently across study groups. For example, the authors reported that DEHP exposures were static, nonrenewal lethality tests, and <i>Daphnia magna</i> adults were placed in a 100-mL glass beaker containing 100 mL of test solution.
Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured and were similar to nominal concentrations, except for nominal concentration 0.06 mg/L for which the authors reported 0.00581 mg/L (given that the rest of the measured concentrations were very similar to the nominal concentrations, it is unclear if the reported value of 0.00581 mg/L was a typo with an extra 0). Moreover, there are no details of when the measured concentrations were taken, and it appears that they were not taken repeatedly such as at the beginning, throughout, and at the end of the study.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (48 h) was reported and suitable for the study type (acute toxicity in <i>Daphnia magna</i> ).
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Adults (>96 h): The number of exposure groups and spacing of exposure levels were suitable for a dose response: 0.2, 0.3, 0.4, 0.5, and 0.6 mg/L and a blank control and a solvent control (0.004% acetone, v/v).

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<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5498837			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility limit for DEHP in the absence of a solvent is 0.270 mg/L. In this study, the authors used a solvent concentration of 0.004% acetone (v/v) to dissolve DEHP. Moreover, the authors reported that preliminary experiments and previous literatures confirmed that acetone at this concentration did not affect survival or biochemical responses in <i>Daphnia magna</i> .
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were obtained from a reliable source and were appropriate [e.g., species ( <i>Daphnia magna</i> ) and age (adults (> 96 h))] for the study type. Moreover, the authors reported following test guidelines of the OECD Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment culture conditions were adequately described in the "Experimental Materials" section, and there is no evidence that they were not the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects. Five replicates were conducted for each treatment concentration (blank control, solvent control, plus DEHP concentrations). In each replicate, ten <i>Daphnia magna</i> adults were placed in a 100-mL glass beaker containing 100 mL of test solution, and a total of 50 organisms were exposed to each concentration in the DEHP gradient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The authors reported following test guidelines of the OECD (2004), which is Test No. 202: <i>Daphnia</i> sp. Acute Immobilisation Test. In addition, the authors reported that the exposures were static, nonrenewal tests and that the test animals were not fed. However, the authors did not describe specific environmental conditions such as water quality control parameters taken during the test.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (organisms were considered immobile if they would not swim within 15 s after gentle agitation) was reported and adequate for the intended outcome of interest (immobilization to determine acute toxicity, LC50).
	Metric 18:	Consistency of Outcome Assessment	Medium	The outcome assessment protocol was reported, and there is no evidence that it was not assessed consistently across study groups.
Domain 6: Confounding / 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 were no reported differences among the study groups in environmental conditions.
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<b>Study Citation:</b>	Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J. (2018). Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult <i>Daphnia magna</i> . Archives of Environmental Contamination and Toxicology 75(1):145-156.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult		
<b>Health Outcome:</b>	Immobilization		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5498837		
Domain	Metric	Rating	Comments
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described. The lethal concentrations such as LC50 and the 95% confidence limits were calculated by Probit analysis using SPSS 21.
	Metric 22: Reporting of Data	Medium	The authors did not report the raw or mean immobilization results for each treatment group. They instead reported LC50 values at 24 and 48 hours, and they reported the effect/correlation of DEHP concentration and exposure duration on acute toxicity.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The goal of the study was to investigate the relationship between DEHP acute exposure (24-h LC50, 48-h LC50), oxidative stress (lipidperoxidation levels, total antioxidant capacity, superoxide dismutase activity, catalase activity, glutathione-S-transferase activity), and antioxidant gene expression (catalase and glutathione-S-transferase mRNA levels) in <i>Daphnia magna</i> newly hatched juveniles (< 24 h) and adults (> 96 h). This form was used to evaluate the acute toxicity (immobilization) in adults (> 96 h).		
<b>Overall Quality Determination</b>		<b>High</b>	



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 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	Low	Few details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured.
	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	Only one treatment was reported.
	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 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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.
Domain 6: Confounding / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	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 Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . <i>Chemosphere</i> 36(6):1367-1379.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	679904

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ""The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room, and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."
	Metric 17: Outcome Assessment Methodology	High	Mortality was evaluated as immobilization.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****High**

<b>Study Citation:</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . Chemosphere 36(6):1367-1379.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679904			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The DEHP was identified by nomenclature (Table 1a).
	Metric 2:	Test Substance Source	High	The sources were identified (Table 1a).
	Metric 3:	Test Substance Purity	High	"These samples are commercial products with stated purities in excess of 99.5 (w/w)."
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	"Ten replicate beakers each containing 1 <i>Daphnia</i> were used for the control, dispersant control (10 mg/l), and for each phthalate treatment."
	Metric 5:	Negative Control Response	High	No effects were observed in any controls.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	A large amount of surfactant (10x the concentration of phthalate) was used to ensure that the phthalate was suspended in the test solution.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	The authors report that for the chronic tests, the actual recovered concentrations resembled nominal when tested in freshly prepared treatment solution and in solution held for the duration of the experiment (Tables 6 & 7).
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency were appropriate for the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	This is a limit test ("3 orders of magnitude" above solubility limit, no adverse effects noted in any exposure). Though DEHP was studied at two concentrations (0.25 mg/L and 1 mg/L) in chronic tests, both concentrations are far above the solubility limit.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations exceeded the solubility limit but were within the dispersibility limit with the dispersants used, as noted by the authors.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"The test organism was the freshwater crustacean <i>Daphnia magna</i> Strauss, derived from continuous laboratory cultures."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	"The cultures were maintained under similar conditions (water, temperature, photoperiod, and feeding) to those described for the reproduction test."
	Metric 15:	Number of Organisms and Replicates per Group	N/A	This was a limit test.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . <i>Chemosphere</i> 36(6):1367-1379.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679904			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	”The reconstituted water medium used for testing and maintenance of stock cultures was Elendt’s M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ””The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room, and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period.”
	Metric 17:	Outcome Assessment Methodology	High	”From day 5, observations were also made daily for the presence of offspring (termed F1 generation).”
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None			

**Overall Quality Determination****High**

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

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<b>Study Citation:</b>	Brown, D., Croudace, C. P., Williams, N. J., Shearing, J. M., Johnson, P. A. (1998). The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the <i>Daphnia magna</i> . <i>Chemosphere</i> 36(6):1367-1379.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	679904

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	"The reconstituted water medium used for testing and maintenance of stock cultures was Elendt's M4 medium (Elendt and Bias, 1990). The water was aerated for > 2 hours before use and had a nominal pH of 8. ""The test system was maintained at 20 +1°C by housing the test vessels in a temperature controlled room and the photoperiod was controlled to 16 hours light and 8 hours dark with a 15 minute transition period."
	Metric 17: Outcome Assessment Methodology	High	"At the end of the test (21 days), the length of each surviving Po generation <i>Daphnia</i> was measured (apex of helmet to base of spine) using a microscope with a calibrated graticule."
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was not conducted and not typical for this type of test.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****High**



<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334281			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported.	
	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 and are similar to nominal concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.	
	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	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects, but no effect was reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.	
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<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334281

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Though statistical analysis was not conducted, there was no effect seen at any concentration.
	Metric 22: Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334281			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported.	
	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 and are similar to nominal concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.	
	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	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects, but no effect was reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	

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<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334281

Domain	Metric	Rating	Comments
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Though statistical analysis was not conducted, there was no effect seen at any concentration.
	Metric 22: Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334281			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported.	
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured and are similar to nominal concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and/or spacing of exposure levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the test species source.	
	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	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects, but no effect was reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were limited.	

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<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 1. The effect of di-2-ethylhexyl phthalate and diisodecyl phthalate on the reproduction of <i>Daphnia magna</i> and observations on their bioconcentration. Chemosphere 11(4):417-426.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334281

Domain	Metric	Rating	Comments
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response (reproduction) of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Some details of exposure administration were reported, and exposures were 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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	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.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334646

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 / 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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**



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

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<b>Study Citation:</b>	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1334951

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18: Consistency of Outcome 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.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: 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**

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

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<b>Study Citation:</b>	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1334951		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	Knowles, C. O., Mckee, M. J., Palawski, D. U. (1987). Chronic effects of di-2-ethylhexylphthalate on biochemical composition survival and reproduction of daphnia-magna. Environmental Toxicology and Chemistry 6(3):201-208.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1334951		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This is an assessment for the biochemical component analysis.		
<b>Overall Quality Determination</b>		<b>High</b>	

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

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



<b>Study Citation:</b>	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to <i>Daphnia magna</i> and rainbow trout ( <i>Oncorhynchus mykiss</i> ). Environmental Toxicology and Chemistry 14(11):1967-1976.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	680120; Linked HERO ID(s): 1316195, 680120			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity was reported as >95%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using a concurrent negative control.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.	
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<b>Study Citation:</b>	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to <i>Daphnia magna</i> and rainbow trout ( <i>Oncorhynchus mykiss</i> ). Environmental Toxicology and Chemistry 14(11):1967-1976.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	680120; Linked HERO ID(s): 1316195, 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 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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	None		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to <i>Daphnia magna</i> and rainbow trout ( <i>Oncorhynchus mykiss</i> ). Environmental Toxicology and Chemistry 14(11):1967-1976.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	680120; Linked HERO ID(s): 1316195, 680120			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity was reported as >95%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using a concurrent negative control.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response by study authors.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	Rhodes, J. E., Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). Chronic toxicity of 14 phthalate esters to <i>Daphnia magna</i> and rainbow trout ( <i>Oncorhynchus mykiss</i> ). Environmental Toxicology and Chemistry 14(11):1967-1976.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	680120; Linked HERO ID(s): 1316195, 680120		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043468			
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.	
	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.	
	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	Reporting omissions are unlikely to have a substantial impact on results.	
	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	Low	Only one exposure group was reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates were suitable.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Details of the environmental conditions of the test system were not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.	
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<b>Study Citation:</b>	Seyoum, A., Pradhan, A. (2019). Effect of phthalates on development, reproduction, fat metabolism and lifespan in <i>Daphnia magna</i> . Science of the Total Environment 654:969-977.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5043468

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcome.

Additional Comments: This evaluation was for Daphnid length.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to <i>Daphnia magna</i> with cover letter dated 032585. :95.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316195; Linked HERO ID(s): 1316195, 680120			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name (DEHP), but no further information was provided.	
	Metric 2: Test Substance Source	High	The test substance was from General Electric Company, Hudson Falls, New York.	
	Metric 3: Test Substance Purity	Low	The purity was not provided.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used (not a solvent control).	
	Metric 5: Negative Control Response	Medium	Authors reported for DEHP that animals in the controls appeared to be trapped at the surface. While the authors claimed that this did not affect the survival of the controls, as evidenced by the <10% mortality in the controls.	
	Metric 6: Randomized Allocation	Medium	Organisms were allocated in an unbiased manner.	
Domain 3: Exposure Characterization				
	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 Administration	High	The exposure was administered consistently across groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured weekly.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was appropriate - 21 days.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	5 concentrations were tested.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Some concentrations exceeded the water solubility limit. An enhanced mixing technique was used to deliver the chemical in the flow-through system.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the <i>Daphnia</i> was Springborn Bionomics.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There is no description of how many replicates there were per treatment or how many animals there were per replicate. An old protocol is cited: "Protocol for conducting chronic toxicity tests with the water flea <i>Daphnia magna</i> )" developed at EG&G Bionomics {1982}.	
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<b>Study Citation:</b>	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to <i>Daphnia magna</i> with cover letter dated 032585. :95.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1316195; Linked HERO ID(s): 1316195, 680120		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The environmental conditions were recorded and were consistent.
	Metric 17: Outcome Assessment Methodology	High	Mortality was assessed weekly, and reproduction was assessed as the cumulative number of offspring.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Authors state that daphnia in the control and 0.29 mg/L exposure group were observed to be entrapped at the surface, which likely affected survival.
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors state that daphnia in the control and 0.29 mg/L exposure groups were observed to be entrapped at the surface, which likely affected survival.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical tests were performed (not well described, authors cite an old method), and all data are presented in the tables for 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			
<b>Overall Quality Determination</b>		<b>High</b>	



<b>Study Citation:</b>	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to <i>Daphnia magna</i> with cover letter dated 032585. :95.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316195; Linked HERO ID(s): 1316195, 680120			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name (DEHP), but no other information was provided.	
	Metric 2: Test Substance Source	High	The test substance was from General Electric Company, Hudson Falls, New York.	
	Metric 3: Test Substance Purity	Low	The purity was not provided.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used (not a solvent control).	
	Metric 5: Negative Control Response	Medium	Authors reported for DEHP that animals in the controls appeared to be trapped at the surface, but overall survival of controls was acceptable.	
	Metric 6: Randomized Allocation	Medium	Organisms were allocated in an unbiased manner.	
Domain 3: Exposure Characterization				
	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 Administration	High	The exposure was administered consistently across groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured weekly.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was appropriate - 21 days.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	5 concentrations were tested. There were no effects on reproduction observed at the highest concentration (at the solubility limit).	
	Metric 12: Testing at or Below Solubility Limit	Medium	Some concentrations exceeded the water solubility limit. An enhanced mixing technique was used to deliver the chemical in the flow-through system.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the <i>Daphnia</i> was Springborn Bionomics.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There is no description of how many replicates there were per treatment or how many animals there were per replicate. An old protocol is cited: "Protocol for conducting chronic toxicity tests with the water flea <i>Daphnia magna</i> )" developed at EG&G Bionomics {1982}.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were recorded and were consistent.	
	Metric 17: Outcome Assessment Methodology	High	Reproduction was assessed as the cumulative number of offspring.	

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<b>Study Citation:</b>	Bionomics,, Springborn (1984). Chronic toxicity of fourteen phthalate esters to <i>Daphnia magna</i> with cover letter dated 032585. :95.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316195; Linked HERO ID(s): 1316195, 680120			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Authors state that daphnia in the control and 0.29 mg/L exposure groups were observed to be entrapped at the surface.	
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors state that daphnia in the control and 0.29 mg/L exposure groups were observed to be entrapped at the surface, which likely affected survival.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical tests were performed (not well described, authors cite an old method), and all data are presented in the tables for the treatments and controls.	
	Metric 22: Reporting of Data	High	All data are presented in the tables for the treatments and controls.	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily described.	
Additional Comments:	None			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pseudolimnaeus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.
Domain 3: Exposure Characterization				
	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 the 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 the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the number of exposure groups and the spacing of 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				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pseudolimnaeus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were 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 / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	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		

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pseudolimnaeus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 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	Low	Few details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured.
	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	Only one treatment was reported.
	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 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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pseudolimnaeus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.
Domain 6: Confounding / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	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 Quality Determination****Uninformative**



<b>Study Citation:</b>	Oil., Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp <i>Gammarus pulex</i> (L) to toxicants with cover letter.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335277			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	DEHP was identified by name only in the report, but the CAS number was given on the cover sheet.
	Metric 2:	Test Substance Source	High	The source is listed as "Hopkins and Williams D.K."
	Metric 3:	Test Substance Purity	High	The purity is listed as "Analar", presumably this means analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were included in the study.
	Metric 5:	Negative Control Response	High	Control mortality is reported as less than 4 percent.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	This was a static renewal test carried out in 350-mL Pyrex glass dishes.
	Metric 8:	Consistency of Exposure Administration	High	Exposure solution was renewed daily.
	Metric 9:	Measurement of Test Substance Concentration	Low	Measurements were not conducted; only nominal concentrations were reported.
	Metric 10:	Exposure Duration and Frequency	High	This was a 96-hour acute toxicity study with a 24-hour post-study depuration.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	The number of concentrations tested is not legible in the scanned copy; the range is given as 1-10 mg/L. It appears that it may be 5 concentrations.
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	All concentrations exceeded the solubility limit of DEHP (0.003 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	G. pulex were collected from a tributary of the River Len at Holingbourne, Kent. Because they were collected from the wild rather than reared in laboratory culture, there are minor reservations about the choice of test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatization was conducted for 7 days.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms were placed in each exposure vessel, with two replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the organisms in question.
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<b>Study Citation:</b>	Oil., Shell (1982). The effects of water hardness, temperature and size of test organism on the susceptibility of fresh water shrimp <i>Gammarus pulex</i> (L) to toxicants with cover letter.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335277			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed by observing appendage movement for 15 seconds.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed simultaneously and in the same way among all 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	High	LC50 values were calculated by probit analysis.
	Metric 22:	Reporting of Data	High	Data for all outcomes were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexplained outcomes.
Additional Comments:	This study was unacceptable because all concentrations of DEHP were well above the solubility limit.			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod <i>Gammarus pulex</i> . Bulletin of Environmental Contamination and Toxicology 46(1):159-166.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Adult
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 / 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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The results were highly variable with no clear trends.

Additional Comments: None

**Overall Quality Determination****Medium**

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

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Medium	Details regarding the execution of the study protocol for outcome assessment were not reported.
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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was not conducted, which is typical for BCF assessments.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod <i>Gammarus pulex</i> . Bulletin of Environmental Contamination and Toxicology 46(1):159-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	732821			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Medium	The chemical was only identified as DEHP, a phthalate ester.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control.	
	Metric 5: Negative Control Response	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. There was some concern over using plexiglass tanks with phthalates.	
	Metric 8: Consistency of Exposure Administration	Medium	Reporting omissions regarding quality checks are likely to have an impact on results.	
	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 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 Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations about the source of test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Replicates were not used or reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health, and biomass loading was appropriate.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.	

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<b>Study Citation:</b>	Thurén, A., Woin, P. (1991). Effects of phthalate esters on the locomotor activity of the freshwater amphipod <i>Gammarus pulex</i> . Bulletin of Environmental Contamination and Toxicology 46(1):159-166.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Gammarus pulex</i> ; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	732821		
Domain	Metric	Rating	Comments
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 in animal attrition.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not 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**



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

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

**Overall Quality Determination****Uninformative**

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hexagenia bilineata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	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 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	Low	Few details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured.
	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	Only one treatment was reported.
	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 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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly reported.

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hexagenia bilineata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334646			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Few details regarding the execution of the study protocol for outcome assessment were provided.
Domain 6: Confounding / 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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	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 Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679311			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The chemical was identified by name. Further details, such as CASRN, were provided in Call et al 2001.	
Metric 2:	Test Substance Source	High	The source of chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).	
Metric 3:	Test Substance Purity	High	Purity was identified as 99% in the cited reference Call et al 2001.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.	
Metric 5:	Negative Control Response	High	The biological response of the control group was appropriate, as shown in Table 5.	
Metric 6:	Randomized Allocation	Low	Random allocation was not stated.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and addition of sediment to test beakers were described in detail.	
Metric 8:	Consistency of Exposure Administration	High	Exposure consistency was reported and consistent.	
Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods and referenced in Call et al 2001.	
Metric 10:	Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed cited methods (EPA, 1994).	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).	
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via sediment.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not provided.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation of test organisms prior to exposure was not reported.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.	

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	679311		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10, and conductivity was measured on days 1 and 9.
	Metric 17: Outcome Assessment Methodology	Medium	The survivor count was determined after the 10 day exposure, but it was not reported as percent mortality.
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups that could influence outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were 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	High	Survival data from toxicity tests were summarized using the trimmed Spearman-Kärber method. Dry weight data were analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStat Program.
	Metric 22: Reporting of Data	Medium	Data were reported in Table 5, however results were pooled among replicates.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.
Additional Comments: None			
<b>Overall Quality Determination</b>		<b>High</b>	



<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	679311		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The chemical was identified by name. Further details, such as CASRN, were provided in Call et al 2001.
	Metric 2: Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3: Test Substance Purity	High	Purity was identified as 99% in the cited reference Call et al 2001.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5: Negative Control Response	High	The biological response of the control group was appropriate as shown in Table 5.
	Metric 6: Randomized Allocation	Low	Random allocation was not stated.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and addition of sediment to test beakers were described in detail.
	Metric 8: Consistency of Exposure	High	Exposure consistency was reported and consistent.
	Metric 9: Administration Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods and referenced in Call et al 2001.
	Metric 10: Exposure Duration and Frequency	High	The duration (10 day exposure) was appropriate for the experimental design, and it followed the cited methods (EPA, 1994).
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	There was one exposure concentration for DEHP in sediment (3000 mg/kg).
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via sediment.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not provided.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimation of the test organisms prior to exposure was not reported.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Tests with DEHP, DINP and DIDP utilized 5 replicates of 3000 mg/kg sediment with 10 organisms per beaker and five sediment control replicates with 10 test organisms per beaker and two silica sand control replicates with 10 test organisms per beaker.
Domain 5: Outcome Assessment			
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyalella azteca</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679311			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10, and the conductivity was measured on days 1 and 9.	
	Metric 17: Outcome Assessment Methodology	High	The sediment was sieved. Survivors were then collected, dried, and weighed.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome was assessed at the conclusion of the 10-day exposure.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among study groups that could influence outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were 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	High	Survival data from toxicity tests were summarized using the trimmed Spearman–Kärber method. Dry weight data were analyzed by one-way analysis of variance and Dunnett’s procedure using a SigmaStat Program.	
	Metric 22: Reporting of Data	High	Treatment and control data were reported in Table 5. Results were represented as average dry weight per individual.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variability was not reported among replicates, but based on information in Table 5, it did not appear that excessive variability occurred.	
Additional Comments:	None			
Overall Quality Determination		High		

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyaella azteca</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.	
	Metric 2: Test Substance Source	High	The source of the phthalate was Aldrich Chemical. The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	The exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.	
	Metric 10: Exposure Duration and Frequency	Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Hyaella azteca</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.	
	Metric 18: Consistency of Outcome Assessment	High	The 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	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.	
Additional Comments:	None			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Lumbriculus variegatus</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.	
	Metric 2: Test Substance Source	High	The source of the phthalate was Aldrich Chemical. The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	The exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.	
	Metric 10: Exposure Duration and Frequency	Low	The duration of exposure and exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of exposure levels were not adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	A subset of the exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Lumbriculus variegatus</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679312			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest, but it wasn't sensitive to the intended endpoint.	
	Metric 18: Consistency of Outcome 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	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were unexpected outcomes with possible explanations.	
Additional Comments:	None			

**Overall Quality Determination****High**



<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by correct nomenclature and chemical structure. The CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source of DEHP was Aldrich Chem. Co.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	The biological responses of the controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure 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 Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	The exposure concentrations were not reported/measured.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to assess cell death (necrosis and apoptosis) and cell morphology impacts in the hemocytes.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentration (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The concentrations were below solubility limit as reported. But, the authors did not report the concentration of acetone used to dissolve the phthalate, and they did not measure the phthalate concentration.
Domain 4: Test Organism				
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
	Metric 13:	Test Organism Characteristics	Medium	The test organisms (freshwater prawn) were obtained from a market, and the hemolymph drawn from these test organisms was then used to isolate the hemocytes employed in the in vitro exposure and assays. Information on the size/age of the prawns was not provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 C for 3 days prior to experiments, and stocking densities were maintained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect the immune system. The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.
	Metric 15:	Number of Organisms and Replicates per Group	Low	This was an in vitro study to determine impacts on hemocytes (immune cells) measured by cell death (necrosis and apoptosis) and cell morphology. The number of organisms used (5-10) to collect a diverse pool of hemocytes and cell suspensions was reported. However, authors offered insufficient details on, for example, the number of replicates examined by gel electrophoresis or the number of sections and replicate fields for the electron microscopy assessment.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	This was an in vitro exposure study to determine impacts on hemocytes measured by cell death (necrosis and apoptosis) and cell morphology. In vitro exposure conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies using hemocytes [the annexin assay, gel electrophoresis, transmission electron microscopy] were reported and appropriate for the outcomes of interest in hemocytes [cell death by necrosis and apoptosis and cell morphology changes].
	Metric 18:	Consistency of Outcome Assessment	High	Details of the in vitro assays to examine hemocyte toxicity/viability were reported and assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared in adequate medium conditions and treated consistently.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Presentation and Analysis				
	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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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	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)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789598

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	Low	Authors did not report SE, SD, CI or such variability details for the cell death (necrosis and apoptosis) or the cell morphology data.

Additional Comments: This study 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 hemocyte toxicity/viability due to DEHP.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The chemical was identified by correct nomenclature and chemical structure. The CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source of DEHP was Aldrich Chem. Co.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	The biological responses of the controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure 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 Administration	High	The exposure was consistent.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported/measured.
	Metric 10:	Exposure Duration and Frequency	High	The exposure time was appropriate.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentrations (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or in only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	All concentrations were below the solubility limit as reported. But, the authors did not report the concentration of acetone used to dissolve the phthalate, and they did not measure the phthalate concentration.
Domain 4: Test 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.
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 C for 3 days prior to experiments, and stocking densities were maintained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect immune function (i.e., stress can modulate immune responses (cell-mediated and humoral). The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	This was an in vitro study to determine impacts on nonspecific cell-mediated immune defense responses. The number of organisms used (5-10) to collect a diverse pool of hemocytes, number of cells and cell suspensions, number of well-plates, and number of replicate fields for microscopic counts were sufficient.
Domain 5: Outcome Assessment				
	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 pseudopodia 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 of in vitro assays were reported and assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	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 the exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was reported but not explained in detail.
	Metric 22:	Reporting of Data	High	Data were reported for each assay and for all treatment groups per assay. The summary of the effects in Table 1 reported the inhibition or enhancement concentration for each assay.
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean for the nonspecific immune response data: hemocyte adhesion and pseudopodia formation, phenoloxidase activity, and superoxide production (Figures 2 and 3).
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	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)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789598

Domain	Metric	Rating	Comments
Additional Comments:	This study was an in vitro experiment where hemocytes (immune cells) isolated from the hemolymph of 5 to 10 giant freshwater prawn ( <i>Macrobrachium rosenbergii</i> ) 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 DEHP.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Paratanytarsus parthenogenetica</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The rest substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. The manufacture name and batch number not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Paratanytarsus parthenogenetica</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
	Metric 17: Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**



<b>Study Citation:</b>	Monsanto, (1983). Acute toxicity of di-(2-ethylhexyl) phthalate (DEHP) to the midge <i>Paratanytarsus parthenogenetica</i> .			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Paratanytarsus parthenogenetica</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1335357			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Medium	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The source of DEHP was R. H. Mills, Plasticizers Division, Monsanto, St. Louis, Missouri. Additional information such as the lot number was accounted for in the log book and in the paper. It was not verified analytically.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were adequate.	
	Metric 6: Randomized Allocation	Medium	Researchers state that 10 midge were randomly assigned to each test vessel.	
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	Details of exposure administration were reported, but minor inconsistencies in administration of exposures among study groups were identified that are unlikely to have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	A standard duration was used.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Low	The concentrations used in the study ranged from 0.62-10 mg/L; all exceeded the limit of solubility.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	

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

**Overall Quality Determination****Low**

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

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

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

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

**Overall Quality Determination****Uninformative**

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

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

**Overall Quality Determination****Uninformative**



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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in <i>Chlorella vulgaris</i> . Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 42(2):179-183.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679344			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design				
	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 the test substance.
	Metric 5:	Negative Control Response	Medium	A baseline control response was noted on graphs and in equations.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	High	Exposures were consistently administered to test organisms.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations were measured but only reported as log values on a graph.
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency differed significantly from typical study designs. Concentrations never reached steady state.
	Metric 11:	Number of Exposure Groups/ 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 Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	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 replicates was not reported.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Chi, J., Li, B., Wang, Q. Y., Liu, H. (2007). Influence of nutrient level on biodegradation and bioconcentration of phthalate acid esters in <i>Chlorella vulgaris</i> . Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering 42(2):179-183.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679344			
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 Assessment	High	Details of the outcome assessment protocol were reported, and 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 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 21:	Statistical Methods	N/A	Only one concentration was used.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment over time.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments:	This evaluation was for DEHP exposure at various N/P levels.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga <i>Chlorella vulgaris</i> . IOP Conference Series: Earth and Environmental Science 227(5):052054.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5692135			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by name and CAS no.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure), but it was unclear whether it was a water or solvent control.	
	Metric 5: Negative Control Response	High	The biological response of the control group was adequate.	
	Metric 6: Randomized Allocation	Low	This is an algal study and reporting of random allocations is limited.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance during exposure. Concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance 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 and outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for the purpose of the study.	
	Metric 12: Testing at or Below Solubility Limit	Low	A solvent was used to prepare test solutions, but it was unclear whether the control had a solvent or not. The control response was adequate. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was reported, and the test organisms were appropriate for evaluation of the specific outcome of interest.	
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<b>Study Citation:</b>	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga <i>Chlorella vulgaris</i> . IOP Conference Series: Earth and Environmental Science 227(5):052054.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5692135			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether the pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The test vessels were inoculated at an initial concentration of 1.2×10^6cells/mL. Each test concentration had four replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate and whether differences occurred between control and exposed populations.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies for hydrogen peroxide and malondialdehyde content, and superoxide dismutase and glutathione peroxidase activities were reported.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported. It was not reported whether the biomarker measurements were done at the end of the growth experiment.
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	In the methods section, it was reported that Student's t test was conducted, but this is not an appropriate test when there are more than 2 treatment groups. ANOVA is the appropriate statistical test to compare more than 2 groups and helps to control Type 1 error.
	Metric 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:	It was unclear whether a water control or a solvent control was used. Incorrect statistical analysis was conducted, and data enabling an independent statistical analysis were not provided. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga <i>Chlorella vulgaris</i> . IOP Conference Series: Earth and Environmental Science 227(5):052054.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5692135			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by name and CAS no.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Study authors reported using an appropriate concurrent negative control group (i.e. all conditions equal except chemical exposure), but it was unclear whether it was a water or solvent control.	
	Metric 5: Negative Control Response	High	The biological response of the control group was adequate.	
	Metric 6: Randomized Allocation	Low	This is an algal study and reporting of random allocations is limited.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance during exposure. Concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	Details of the 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	Medium	The duration of exposure (5 days) was reported but was higher than the duration recommended by OECD 201 test guidelines (72 hours), and no justification was provided.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for the purpose of the study.	
	Metric 12: Testing at or Below Solubility Limit	Low	A solvent was used to prepare test solutions, but it was unclear whether the control had a solvent or not. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was reported, and the test organisms were appropriate for evaluation of the specific outcome of interest.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether the pretreatment conditions were the same for the control and the exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The test vessels were inoculated at an initial concentration of 1.2×10^6cells/mL. Each test concentration had four replicates.	
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<b>Study Citation:</b>	Shen, C., Wang, Y., Shen, Q.,i, Wang, L.,i, Lu, Y., Li, X.,in, Wei, J.,ie, IOP (2019). Di-(2-ethylhexyl) phthalate induced the growth inhibition and oxidative damage in the microalga <i>Chlorella vulgaris</i> . IOP Conference Series: Earth and Environmental Science 227(5):052054.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	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)		
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5692135		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate and whether differences occurred between control and exposed populations.
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. It was stated the algal cell density of the algal culture was determined using a Neubauer haemocytometer, but the methods were not provided.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported.
Domain 6: Confounding / 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 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was not conducted for the growth experiment data, and data enabling an independent statistical analysis were not provided. A 96h EC 50 value was reported, but the statistical method used was not reported. Mean algal density data was given in the figure without any measure of variability. In the methods section, it was reported that Student's t test was conducted, but this is not an appropriate test when there are more than 2 treatment groups.
	Metric 22: Reporting of Data	Low	Cell density data was presented without measures of variability. Specific growth rate was calculated but not reported. The 96H EC value was reported without confidence intervals.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals), and insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.
Additional Comments:	It was unclear whether a water control or a solvent control was used. Statistical analysis was not conducted for the growth experiment and data enabling an independent statistical analysis were not provided. The solubility limit was exceeded in all treatment concentrations (2,4,6,8,10 mg/L) according to the solubility listed in the final scope (0.27 mg/L).		

**Overall Quality Determination****Low**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Pseudokirchneriella subcapitata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789536			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The test substance was identified definitively. Nomenclature, CASRN, and structure were reported.	
Metric 2:	Test Substance Source	High	DEHP was purchased from Merck Eurolab (Stockholm, Sweden), but the test substance identity was NOT analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	High	Percent purity was reported as >98%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
Metric 5:	Negative Control Response	High	The biological response of the control group was reported. The control group had a growth rate 1.6-1.8/ d during the 72 hour of incubation.	
Metric 6:	Randomized Allocation	Low	There were minor limitations in the allocation method that are unlikely to have a substantial impact on results. This was an algal study, and reporting of random allocations are limited.	
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 and/or minimize loss of the test substance before and during the exposure.	
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 but 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. The algal growth inhibition test was conducted for 72 hours.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information is provided on the exposure concentrations or on 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 Organism				
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.	

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Pseudokirchneriella subcapitata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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 Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were conducive to the maintenance of health. Typical control growth rates of 1.6-1.8/d were observed in controls.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology was reported. The cited reference (Mayer et al. 1997) has the detailed methodology.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups. Samples were taken from each test flask and the controls every 24 h to determine growth rates.
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Nonlinear regression analysis was conducted to estimate EC values and confidence intervals.
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. Only the EC 10 and EC 50 values were provided.
	Metric 23:	Explanation of Unexpected Outcomes	Low	There were no unexpected outcomes.
Additional Comments:	The exposure concentrations, spacing of exposure levels, and control response were not reported. Measured concentrations were not reported. Growth rate data were not provided for each of the treatment groups and control. Only EC 10 and EC 50 values were reported.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency of the exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	The environmental conditions were appropriate for the test.
	Metric 17: Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**

<b>Study Citation:</b>	Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga <i>Selenastrum capricornutum</i> .			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316196			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name and CASRN.	
	Metric 2: Test Substance Source	Low	The source of the chemical was not included in the technical report.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade were not included in the technical report.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were included in the study.	
	Metric 5: Negative Control Response	High	There was an adequate response from the negative controls.	
	Metric 6: Randomized Allocation	Medium	The allocation of algae for the experiments was not described in the study.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The study followed OECD 201 (1981) guidelines and included any deviations.	
	Metric 8: Consistency of Exposure Administration	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 chromatography with electron capture detection. Concentrations were measured throughout the 10 days. The initial and final concentrations can be found in the text.	
	Metric 10: Exposure Duration and Frequency	High	The 10-day exposure was adequate for the study.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	There was an appropriate number of exposure groups.	
	Metric 12: Testing at or Below Solubility Limit	High	No response was observed for the single concentration recorded below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source and the details of the algae were limited.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Acclimatization details were limited.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The study followed OECD 201 (1981) guidelines using adequate numbers of organisms.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Test conditions were reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome for the chemical was described in detail in results.	
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<b>Study Citation:</b>	Bionomics,, Springborn (1984). FYI Submission: Toxicity of fourteen phthalate esters to the freshwater green alga <i>Selenastrum capricornutum</i> .			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316196			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were reported consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No differences were reported among the study groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistics used include, moving average angle analysis, probit analysis, and binomial probability.	
	Metric 22: Reporting of Data	Medium	Data was reported for growth.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	Initial studies to determine test concentrations concluded that no response from the algae was observed at concentrations below the water solubility limit, therefore 1 concentration (below the solubility limit) was reported with no response.			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Uninformative**



<b>Study Citation:</b>	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lemna minor</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1340050			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Reporting omissions were unlikely to have a substantial impact on results.
	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 the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Minor uncertainties were identified regarding environmental conditions, but these are not likely to have a substantial impact on results.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.

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

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

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

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Xu, G., Wu, M. H., Zheng, J. F., Jiao, Z., Li, F. S. (2008). Aquatic toxicity of di (2-ethylhexyl) phthalate (DEHP) to duckweeds. :978-981.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Spirodela polyrhiza</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1340050			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	the chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Reporting omissions were unlikely to have a substantial impact on results.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism				
	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.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Minor uncertainties were identified regarding environmental conditions, but these are not likely to have a substantial impact on results.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.

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

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
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 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	
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				
	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 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 the exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the biological response of the solvent control was acceptable, and no interactions are expected between the solvent and test substance.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3515118

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health. The germination experiment was conducted in "a growth chamber in total darkness at temperature of $25 \pm 1^{\circ}\text{C}$ 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 was not described adequately.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described well.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, for germination rate (Fig 1) and root/shoot elongation (Fig 2). IC 10 and IC50 values were provided for root and shoot elongation.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation form is relevant for germination rate and shoot/root growth.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
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 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	
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				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the test media preparation methods were adequately reported. The test solutions were replenished daily.
	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 the spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the biological response of the solvent control was acceptable.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health. "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 surface area, average root diameter, and the number of root tips and hairs) were obtained from digital images.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / 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 exposure (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 2 and Fig 3).	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	Root morphology measurements included total root length, total root surface area, average root diameter, and the number of root tips and hairs. The exposure concentrations were not verified.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
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 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	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	Medium	The experimental system and the test media preparation methods were adequately reported. The test solutions were replenished daily.	
	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 the 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 not the biological response of the solvent control.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health. "The experiments were performed in an artificial climate chamber with alternating temperatures of 25 ± 1oC (12 h light) and 20 ± 1oC (12 h dark), 60% relative humidity, and a light intensity of 40 uM/ms".	
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology (for determining DBP and DEHP in shoot and root) reported the intended outcome of interest. Key details for HPLC analysis (method detection limit, percent recovery , etc.) were not provided.	
	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 exposure that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment group. The control group response was not reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	The control group was not analyzed for DEHP. The exposure concentrations were not verified.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
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 source was reported as J&K Scientific Ltd. (Beijing, China), but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Chemical purity was reported as 96.8%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and the test media preparation methods were adequately reported. The test solutions were replenished daily.	
	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 the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The solvent concentration slightly exceeded an appropriate concentration, but the biological response of the solvent control was acceptable, and no interactions are expected between the solvent and test substance.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp.</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3515118			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health. "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	Low	The outcome assessment methodologies for enzyme activities, lipid peroxidation and permeability of plasma membrane were not clearly reported.	
	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 exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group 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 Quality Determination		High		

<b>Study Citation:</b>	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows ( <i>Cyprinodon variegatus</i> ). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	18110; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was not reported.	
	Metric 3: Test Substance Purity	High	All the chemicals used in this study were reported to be analytical grade with a minimum purity of 80%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using negative controls that contained any of the solvents or carriers at the highest levels used in the test concentrations.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported.	
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Tests were reported to be static tests. The test solution was prepared by either adding the appropriate amount of weighed test substance to each test container, or by adding the appropriate volume of test substance and solvent/carrier to each test container.	
	Metric 8: Consistency of Exposure Administration	Medium	All tests were for 96h. Study authors reported using two different sized test containers, a 4L container size and a 19L container size. This would most likely not have a substantial impact on the results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Study authors did not report if exposure concentrations were measured.	
	Metric 10: Exposure Duration and Frequency	High	This was a 96h acute toxicity test, which is typical for fish species.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The study authors did not report the number of exposure groups or the spacing of the exposure groups. Only LC50 values were reported. However, the cited methods (EPA 1975) specifies that "for determination of an LC50 or an EC50, a control and at least five concentrations of toxicant in a geometric series should be used."	
	Metric 12: Testing at or Below Solubility Limit	Low	Study authors did not report the exposure concentrations used, so it is unable to be determined if the exposure values were below the solubility limit. It was reported that solvents/carriers were used when necessary.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Test organisms were hatched and reared at BMRL and were originally from EPA in Gulf Breeze, FL. Juvenile organisms were used for the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions in any way.	
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<b>Study Citation:</b>	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows ( <i>Cyprinodon variegatus</i> ). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	18110; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test chamber. The number of replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Organisms were fed until the start of the study. Before the start of the study, they were kept at 25-31 C in seawater with ambient salinity. The actual temperature during the study was not reported.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–LC50 values/mortality.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Organisms were checked for mortality every 24h for 96h.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated in any way.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were adequate and described in the text.	
	Metric 22: Reporting of Data	Low	Only LC50 values were reported. There was no raw data reported for mortalities in test concentrations or controls.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.	
Additional Comments:	This portion of the evaluation is on the effect of diethethylhexyl phthalate on juvenile sheepshead minnows. LC50 values were reported, so mortality was selected as the outcome.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519010			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.	
Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the substance identity was verified.	
Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water.	
Metric 5:	Negative Control Response	High	The control responses were adequate.	
Metric 6:	Randomized Allocation	Low	Researchers did not state if the fish were allocated at random.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	DEHP was dissolved in dimethyl sulfoxide. The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure. Concentrations were not measured.	
Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to examine growth and reproductive effects (6 months).	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1 mg/L of DEHP, 0.5 mg/L of DEHP.	
Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected between the solvent and test substance)).	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the exposure was as follows: "Embryos were collected from the abdomens of healthy females, and the larvae hatched within one week were used for the subsequent exposure experiment."	
Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.	

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

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519010			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.
	Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the substance identity was verified.
	Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water.
	Metric 5:	Negative Control Response	High	The control responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not state if the fish were allocated at random.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Test media preparation details were not provided other than DEHP was dissolved in dimethyl sulfoxide. Concentrations were not measured.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration ( 6 months) was appropriate to examine mechanistic effects.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1 mg/L of DEHP, 0.5 mg/L of DEHP.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were near the solubility limit for DEHP, but an appropriate solvent was used. The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected between the solvent and test substance)).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the exposure was as follows: "Embryos were collected from the abdomens of healthy females, and the larvae hatched within one week were used for the subsequent exposure experiment."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.

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<b>Study Citation:</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519010			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	50 fish per replicate were used, and there were 3 replicates per treatment group.For VTG analyses 5 fish livers were pooled. Pooling replicates is not best practice because it makes it impossible to capture variation across individual replicate animals.For RNA 3 replicates were used, which is a very small sample size for gene expression work. For E2 and T concentrations, 5 individuals per sex were used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Housing and environmental conditions were appropriate for the marine medaka. The fish were raised in artificial seawater under standard laboratory conditions of 28 ± 1 °C on a 14:10 light/dark photoperiod. The fish were fed with freshly hatched <i>Artemia</i> nauplii twice daily.	
	Metric 17: Outcome Assessment Methodology	High	The endocrine endpoints examined (E2 and testosterone concentrations, expression of hypothalamic pituitary gonadal axis genes as well as VTG) were sensitive and appropriate.	
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment methods were reported and were the same across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Information that could be used to compare environmental conditions across groups (measured concentrations, monitoring data for pH, DO, etc.) was limited.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information suggested differences occurred that were unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The authors used one-way ANOVAs and Tukey’s post hoc tests.	
	Metric 22: Reporting of Data	High	Means and SEM for both treatments and the control were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	The authors do not describe any unexpected outcomes.	
Additional Comments:	The study examines the effects of 6 months of exposure to DEHP, and evaluates a number of apical and mechanistic endpoints in the marine medaka. This evaluation form is relevant to the mechanistic effects examined (E2 and testosterone concentrations, expression of hypothalamic pituitary gonadal axis genes as well as VTG).The study does not verify exposure concentrations.			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519010			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	Di-(2-ethylhexyl)-phthalate (DEHP) was identified as one of the test substances. No other information (CASRN, structure, etc.) was provided.	
Metric 2:	Test Substance Source	Low	DEHP was obtained from Supelco (Bellefonte, PA, USA), but it is unknown if the substance identity was verified.	
Metric 3:	Test Substance Purity	Low	The purity of DEHP was not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	A solvent control was used: DMSO at a final concentration of 0.1% in water.	
Metric 5:	Negative Control Response	High	The control responses were adequate.	
Metric 6:	Randomized Allocation	Low	Researchers did not state if the fish were allocated at random.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	Only limited details were given regarding experimental system and test media preparation methods. DEHP was dissolved in dimethyl sulfoxide. Concentrations were not measured.	
Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to examine reproductive effects (6 months).	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	The experiment consisted of only 2 exposure concentrations and the solvent control: 0.1 mg/L of DEHP, 0.5 mg/L of DEHP.	
Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations are near the solubility limit for DEHP, but an appropriate solvent was used. The solvent concentration was appropriate ((i.e., no effects on biological responses were observed in the solvent control, and no interactions were expected between the solvent and test substance)).	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The original source of the fish was not reported. The selection of the animals for the exposure was as follows: "Embryos were collected from the abdomens of healthy females, and the larvae hatched within one week were used for the subsequent exposure experiment."	
Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for treatment and control groups.	
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<b>Study Citation:</b>	Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka ( <i>Oryzias melastigma</i> ). <i>Aquatic Toxicology</i> 146:115-126.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias melastigma</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519010			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	50 fish per replicate were used, and there were 3 replicates per treatment group. To examine reproductive effects, five exposed males or females per replicate were paired with five unexposed females or males.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing and environmental conditions were appropriate for the marine medaka. The fish were raised in artificial seawater under standard laboratory conditions of 28 ± 1 °C on a 14:10 light/dark photoperiod. The fish were fed with freshly hatched <i>Artemia</i> nauplii twice daily.
	Metric 17:	Outcome Assessment Methodology	High	The reproductive endpoints examined (time to start spawning, eggs/female/day, fertilization success (%), sex ratio ( female: male); Table 2) were sensitive and appropriate.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment methods were reported and were the same across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Information that could be used to compare environmental conditions across groups (measured concentrations, monitoring data for pH, DO, etc.) was limited.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested differences occurred that were unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The authors used one-way ANOVAs and Tukey’s post hoc tests.
	Metric 22:	Reporting of Data	High	Means and SEM of reproductive parameters (Table 2) for both treatments and the control were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors do not describe any unexpected outcomes.
<b>Additional Comments:</b>	The study examines the effects of 6 months of exposure to DEHP and evaluates a number of apical and mechanistic endpoints in the marine medaka. This evaluation form is relevant to the reproductive endpoints (Time of start spawning, eggs/female/day, fertilization success (%), Sex ratio ( female: male)).The study does not verify exposure concentrations.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>sheepshead minnow</i> ( <i>Cyprinodon variegatus</i> ); Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316224; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test material was identified, and the CASRN was given.	
	Metric 2: Test Substance Source	High	The source was listed as EG&G Bionomics Aquatic Toxicology Laboratory in Wareham, Massachusetts. No other information about the source was given.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used.	
	Metric 5: Negative Control Response	High	No mortality was reported in the controls.	
	Metric 6: Randomized Allocation	Medium	Test organisms were impartially distributed to each chamber (pdf pg 136).	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were described in adequate detail and accounted for the properties of the test material. For all low-solubility phthalates, an enhanced mixing procedure was used, while for the butyl benzyl phthalate exposure, microbial degradation was accounted for with a cleaning 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 the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Concentrations were measured using GS-MS to account for poor water solubility.	
	Metric 10: Exposure Duration and Frequency	High	A 96 hour exposure was appropriate for an acute test.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Test concentration spacing was limited, as this was designed as a limit test up to the solubility limit of the chemical.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit, which were reported in Appendix A (pdf pg 164).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Specimens were either cultured at the Laboratory or purchased commercially. All fish were tested as juveniles, <10weeks old.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	A 96 hour acclimation period was reported.	

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<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>sheepshead minnow (Cyprinodon variegatus)</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316224; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	Only two replicates of 10 fish were used in each treatment.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and biomass loading was appropriate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes(s) of interest (mortality).	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	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 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:	The 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, butyl benzyl phthalate was referred to as 1D, DIDP was referred to as 1L, and DINP was referred to as 1J.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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 <i>Haliotis diversicolor supertexta</i> . <i>Ecotoxicology</i> 18(3):293-303.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	697762			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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	The chemical purity was reported as at least 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.
	Metric 5:	Negative Control Response	High	The biological responses (percentage of cleavage, normal blastula, larval settlement and metamorphosis) 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	Medium	Methods for preparation of the test media were described in adequate detail; however, steps taken to minimize loss of test substance were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured at the end of the experiment, but it was not clear whether new test solutions were used for the incubation from the swimming stage to the metamorphosis stage. Measured concentrations were similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The total duration of the exposure was 96 hours. The experiment was done in 2 phases covering developmental stages from fertilization to early veliger stage (12 hours) and from veliger to metamorphosis stage (84 hours).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups and the spacing of exposure levels. 96 hr EC-50 values could not be established using the concentrations tested.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration (3.75 %v/v) slightly exceeded an appropriate concentration, but the biological response of the solvent control was acceptable.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations or uncertainties about the choice of the test species source. Test organisms were collected from the field, and prior exposure to phthalates may have occurred.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
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<b>Study Citation:</b>	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 <i>Haliotis diversicolor supertexta</i> . <i>Ecotoxicology</i> 18(3):293-303.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	697762			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and the number of replicates were reported. The fertilized egg density used for each treatment group and controls were not verified after adding 10 ml of fertilization medium to each test vessel.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to the maintenance of organism health. DEHP was found in the dilution medium (1,119 ± 20 ng/l ; Table 2) .
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed the intended outcomes. The percentage of embryos undergoing cleavage and the percentage of settled larvae were not sensitive endpoints. However, the percentage of normal blastula was a sensitive end point, and 9hr EC 50 values were calculated. 96 hr -EC 50 values based percentage of larvae that underwent metamorphosis could not be calculated, instead 96- hr NOEC values were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable 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 outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. ANOVA was used to test for differences among treatments, and probit analysis was used to calculate 9-hr EC 50 values.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes. There was large within study variability for the settlement end point as both normal and abnormal larvae settled.
Additional Comments:	9hr EC 50 values were reported based on the percentage of normal blastula, and 96-hr NOEC values were reported based on the percentage of larvae that underwent metamorphosis. For DEHP, 96-hr NOEC values were adjusted for background concentration of DBP in the dilution medium.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1322103			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	High	The test substance was obtained from Sigma-Aldrich China.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as >99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.
	Metric 5:	Negative Control Response	High	The biological responses of the negative control groups were reported for settlement rate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the 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 Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was to the trochophore stage, which was somewhat arbitrary.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. A solvent aided in dissolution.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.
	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	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.

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<b>Study Citation:</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Larvae
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1322103

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment protocol was reported and consistent 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 outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . <i>Environmental Pollution</i> 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249532			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity was reported as >=98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit, but they were aided by a solvent.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment 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 the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.	

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<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . Environmental Pollution 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249532			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and 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	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:	This evaluation is for gene expression.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . <i>Environmental Pollution</i> 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249532			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity was reported as >=98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate.	
	Metric 12: Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit. They were aided by a solvent.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.	
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<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . Environmental Pollution 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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 / 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	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: This evaluation assessed metamorphosis rate, hatch rate, abnormality rate, and surface structure changes.				

**Overall Quality Determination****High**



<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . <i>Environmental Pollution</i> 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249532			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The purity was reported as $\geq 98\%$ .
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	The experimental system and the methods for preparation of the test media were described in adequate detail.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance 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	High	The number of exposure groups and the spacing of exposure levels were adequate.
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the water solubility limit, but they were aided by a solvent.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcomes of interest.
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<b>Study Citation:</b>	Zhou, J., Cai, Z. H., Xing, K. Z. (2011). Potential mechanisms of phthalate ester embryotoxicity in the abalone <i>Haliotis diversicolor supertexta</i> . Environmental Pollution 159(5):1114-1122.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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 / 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	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: This evaluation is for MDA and POD changes in the organisms.				

**Overall Quality Determination****High**

<b>Study Citation:</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1322103			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The test substance source was Sigma-Aldrich China.	
	Metric 3: Test Substance Purity	High	The test substance purity was reported as >99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using appropriate concurrent negative and solvent control groups.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control groups were reported for abnormal development.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the 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 Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	The exposure duration was to the trochophore stage, which was somewhat arbitrary.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. A solvent aided in the dissolution of the test substance.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.	
	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	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	

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<b>Study Citation:</b>	Yang, Z. H., Zhang, X. J., Cai, Z. H. (2009). Toxic effects of several phthalate esters on the embryos and larvae of abalone <i>Haliotis diversicolor supertexta</i> . Chinese Journal of Oceanology and Limnology 27(2):395-399.
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Haliotis diversicolor supertexta</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 term "abnormal" was not well defined.
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 from outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	The term "abnormal" was not well defined. It was unclear which abnormalities were assessed.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, <i>Macrophthalmus japonicus</i> . Fish and Shellfish Immunology 87:322-332.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Macrophthalmus japonicus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5567571

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 test substance identity was not analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6: Randomized Allocation	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 experimental system was not described well. The study provided only limited details on the measures taken to appropriately prepare the test concentrations.
	Metric 8: Consistency of Exposure Administration	Medium	Few details of the exposure administration were reported. The solvent concentration used was not reported.
	Metric 9: Measurement of Test Substance 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	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.
	Metric 12: Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the biological response of the solvent control was reported and adequate.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	There were concerns regarding the source of the test organisms. Crabs were collected from fish markets, and prior exposure to DEHP may have occurred.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			

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<b>Study Citation:</b>	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, <i>Macrophthalmus japonicus</i> . Fish and Shellfish Immunology 87:322-332.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Macrophthalmus japonicus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5567571			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were adequate and whether differences occurred between control and exposed populations.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was not clearly described.
	Metric 18:	Consistency of Outcome Assessment	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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to the exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	One-way analysis of variance (ANOVA) was conducted combined with Tukey’s multiple range test.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Environmental conditions and exposure concentrations were not measured/reported during the 7 day experiment. Test organism were collected from fish markets and cultured in natural seawater. Background concentration of DEHP in dilution medium was not reported.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, <i>Macrophthalmus japonicus</i> . Fish and Shellfish Immunology 87:322-332.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Macrophthalmus japonicus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5567571			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	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 experimental system was not described well. The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8: Consistency of Exposure Administration	Medium	Few details of the exposure administration were reported. The solvent concentration used was not reported.	
	Metric 9: Measurement of Test Substance 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	High	The number of exposure groups and the spacing of exposure levels were suitable for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the biological response of the solvent control was reported and adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	There were concerns regarding the source of the test organisms. Crabs were collected from fish markets, and prior exposure to DEHP may have occurred.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if they were adequate and whether differences occurred between control and exposed populations.	
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<b>Study Citation:</b>	Park, K., Kim, W. S., Kwak, I. S. (2019). Endocrine-disrupting chemicals impair the innate immune prophenoloxidase system in the intertidal mud crab, <i>Macrophthalmus japonicus</i> . Fish and Shellfish Immunology 87:322-332.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Macrophthalmus japonicus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5567571			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest. Methods for phenoloxidase activity and proPO-related gene expression were given in detail.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed. One-way analysis of variance (ANOVA) was conducted combined with Tukey’s multiple range test.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Phenoloxidase activity, mRNA transcript, and activity levels of six immune-related genes, including lipopolysaccharide and $\beta$ -1,3-glucan-binding protein (LGBP), proPO, phenoloxidase (PO), peroxinectin (PE), serine protease inhibitor (Serp), and trypsin (Tryp), were assessed in gill and in the hepatopancreas of intertidal mud crabs.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The test substance was reported as provided by the manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures taken to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	The exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and the end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of the exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	An appropriate acclimation period for the test was reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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. <i>Environmental Toxicology and Chemistry</i> 14(9):1569-1574.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1321996; Linked HERO ID(s): 1321996, 1316224

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
	Metric 17: Outcome Assessment Methodology	High	The intended outcomes were reported.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Environmental conditions were consistent across groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis			
	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**

<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp ( <i>Mysidopsis bahia</i> ).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316220			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by chemical name. No CASRN or structure were provided.	
	Metric 2: Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA).	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the substance were not included in the study.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Authors reported using negative controls.	
	Metric 5: Negative Control Response	High	The response of the negative controls was adequate.	
	Metric 6: Randomized Allocation	Medium	Mysid shrimp were maintained 1-3 days before they were distributed into test vessels.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental design followed protocol guidelines.	
	Metric 8: Consistency of Exposure Administration	High	Authors reported consistent administration.	
	Metric 9: Measurement of Test Substance Concentration	High	Phthalates were analytically verified and measured.	
	Metric 10: Exposure Duration and Frequency	High	The test duration followed protocol.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	This study only performed a single exposure (0.44 mg/L), as a range finding test reported no effect at levels below the water solubility limit.	
	Metric 12: Testing at or Below Solubility Limit	Low	The single concentration used for DEHP was 0.44 mg/L and is over the solubility listed in the Final Scope (0.27 mg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The source of the organisms was reported. Details beyond that were not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Organisms were housed for 1-3 days prior to treatment.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Replicates followed protocol.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate and described in detail.	
	Metric 17: Outcome Assessment Methodology	High	Outcomes were reported and addressed.	

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<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316220			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed and reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No differences were reported.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	No data analysis was presented as the LC50 was >0.44 mg/L.	
	Metric 22: Reporting of Data	High	Data was reported adequately.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	Based on preliminary studies, concentrations of DEHP below the solubility limit did not result in adverse outcomes; therefore, a single concentration was included in the final study.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 2. The bioconcentration and depuration of di-2-ethylhexyl phthalate and diisodecyl phthalate in mussels, ( <i>Mytilus edulis</i> ). Chemosphere 11(4):427-435.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Mytilus edulis</i> ; Adult			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334379			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Correct nomenclature was given, and the specific form (radiolabeled) was reported.
	Metric 2:	Test Substance Source	High	The source was reported. The radiolabled DEHP was synthesized (by Dr. D Parker, Physics and Radioisotopes Services, ICI PIC,P 0 Box 2, Billingham, England) from phthalic anhydride, and the test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Percent purity was reported as >97.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A concurrent negative solvent control was used.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Experimental system and test media preparation details were adequately reported but did not completely account for physical-chemical properties. A flow-through system was used, and exposure concentrations were measured.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured but methods were not described well.
	Metric 10:	Exposure Duration and Frequency	High	This was a 28 day test to determine BCF.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	The study goal was not to have a dose dependent effect but to determine BCF.
	Metric 12:	Testing at or Below Solubility Limit	Low	The solvent concentration ( 0.5 ml/l) slightly exceeded the typical recommended value ( 0.1 ml/l; OCSPP 850.173: Fish BCF guidelines), and the biological response of the solvent control was not reported.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Mussels were collected locally, and mean shell length and tissue weight of a subset of samples were reported. There was significant concern regarding the source of the mussels. Locally collected mussels could have been exposed to phthalates and a multitude of other stressors prior to the experiment.
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<b>Study Citation:</b>	Brown, D., Thompson, R. S. (1982). Phthalates and the aquatic environment: Part 2. The bioconcentration and depuration of di-2-ethylhexyl phthalate and diisodecyl phthalate in mussels, ( <i>Mytilus edulis</i> ). Chemosphere 11(4):427-435.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Mytilus edulis</i> ; Adult			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1334379			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups. 80 mussels were exposed to the 2 concentrations of DEHP. Replicates were not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Low	
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions (pH, dissolved oxygen, salinity, etc.) were not reported to evaluate if adequate and whether differences occurred between control and exposed populations.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. The methods for tissue concentration analysis were not given in detail. Lipid content in mussels was not determined. Since phthalates are hydrophobic, lipid fraction in mussels should have been determined before and at the end of the uptake experiment and depuration period. This is a serious omission and likely to have a substantial impact on results.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	Low	Data presentation was inadequate. The uptake and depuration rate constants with confidence limits were not reported. Methods for calculating BCF were not given, and BCF values were reported without measures of variability.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
<b>Additional Comments:</b>	Mussels were collected locally and were not acclimatized. Tissue concentrations in controls were not reported. Lipid content in mussels was not analyzed. Uptake and depuration rate constants were not reported. BCF calculations were not provided.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). <i>Water, Air, and Soil Pollution</i> 9(3):323-336.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333217			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	High	The biological response of the solvent control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Differences from nominal values varied considerably.
	Metric 8:	Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via gas chromatography, but measured values were not similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of chemical were noticed in test chambers.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were collected from the wild, with minimal characteristic information given:”Gravid female <i>Palaemonetes pugio</i> 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.
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<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). Water, Air, and Soil Pollution 9(3):323-336.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333217			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	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 in animal attrition.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Some variability was considered to be attributed to incomplete dissolution of the test chemical.
Additional Comments:	None			
Overall Quality Determination		Medium		



<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). <i>Water, Air, and Soil Pollution</i> 9(3):323-336.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333217			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	High	The biological response of the solvent control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Measured values differed from nominal values considerably.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via gas chromatography, but measured values were not similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of the chemical were noticed in the test chambers.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were collected from the wild, with minimal characteristic information given: "Gravid female <i>Palaemonetes pugio</i> 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.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).
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<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). <i>Water, Air, and Soil Pollution</i> 9(3):323-336.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1333217

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. Some variability was considered to be attributed to incomplete dissolution of the test chemical.

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). <i>Water, Air, and Soil Pollution</i> 9(3):323-336.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1333217			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by the accepted name [Di-2-ethylhexyl phthalate (DEHP)].	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response of the solvent control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Differences from nominal values varied considerably.	
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were sampled at 0hr and after 24hr. They were measured via gas chromatography, but measured values were not similar to nominal concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Low	Exposure concentrations were at or below the water solubility limit, however droplets of the chemical were noticed in test the chambers.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There are minor reservations regarding the test organisms. Test organisms were collected from the wild, with minimal characteristic information given:”Gravid female <i>Palaemonetes pugio</i> 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.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects (75 larvae per concentration, three replicates).	
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<b>Study Citation:</b>	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 <i>Palaemonetes pugio</i> (Holthuis). <i>Water, Air, and Soil Pollution</i> 9(3):323-336.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>PALAEMONETES PUGIO</i> ; Larvae
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1333217

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. Some variability was considered to be attributed to incomplete dissolution of the test chemical.

Additional Comments: This form is for the ADME outcome with DEHP.

**Overall Quality Determination**

**Medium**

<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical name was provided with correct nomenclature.	
	Metric 2: Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.	
	Metric 3: Test Substance Purity	High	The source was listed as Sigma Aldrich. Chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Controls for the acute mortality experiment were reported on page 3/8.	
	Metric 5: Negative Control Response	Medium	Control survival for nauplii was reported as 82.6% with an SE of 5.1%.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported for Nauplii.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The acute bioassay was described as a static-renewal test and was detailed for adults in section 2.2.1 (page 2/8).	
	Metric 8: Consistency of Exposure Administration	High	There were 6 treatment concentrations, and they appeared to be consistently applied.	
	Metric 9: Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentrations.	
	Metric 10: Exposure Duration and Frequency	High	A 48 hr exposure duration for these zooplankton is appropriate.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The acute bioassay used 6 concentrations with a control.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit. The highest concentration used on the acute bioassay with nauplii was 1953 ng/L. The final Scope for DEHP lists the solubility for this compound as 2700 ug/L.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The acute bioassays on nauplii were from in house cultures.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors reported 3 replicates per treatment concentration and 60 nauplii per replicate.	

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<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 48 hr bioassay.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology resulted in the formation of a dose-response curve presented in Figure 2.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment within this acute bioassay appeared to be consistent among treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to exposure influenced outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. The authors did not report how the dose-response curve was estimated with survival data. LC 50 concentrations were presented in section 3.1, and a "sigmoidal model" was presented in Figure 2 (page 5/8).
	Metric 22:	Reporting of Data	Low	Raw data for exposure-related findings were not shown for each treatment and control, but adjusted survival data for treatment groups were given in Figure 2. Control survival were reported as a 48 hr mean of the replicates.
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability in survival (%) are reported in section 3.1 as standard error (SE).
Additional Comments:	P. crassirostris nauplii were more sensitive to DEHP than adults. The 48h LC50 value established for nauplii was 1.04ng/L.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical name was provided with correct nomenclature.	
	Metric 2: Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.	
	Metric 3: Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product was >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Controls for the acute mortality experiment were reported on page 3/8. Solvent controls were used for the two highest concentrations of the compound.	
	Metric 5: Negative Control Response	Low	Section 3.1 and supplemental notes (Table S.2) do not report control survival for the adult acute exposures.	
	Metric 6: Randomized Allocation	Medium	Random allocation was reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The acute bioassay was described as a static-renewal test and was detailed for adults in section 2.2.1 (page 2/8).	
	Metric 8: Consistency of Exposure Administration	Low	Half of the 6 highest concentrations contained a 4% ethanol carrier due to solubility concerns. The lowest three treatment concentrations did not contain a solvent carrier. Authors reported using both solvent and non-solvent controls but did not report control survival.	
	Metric 9: Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentrations.	
	Metric 10: Exposure Duration and Frequency	High	A 48 hr exposure duration for these zooplankton is appropriate.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The acute bioassay used 6 concentrations with a control and solvent controls. Authors reported in section 3.1 (page 4/8) that there were no significant differences between treatments and controls for the adult exposures. LC 50 values could not be established at the tested concentrations.	
	Metric 12: Testing at or Below Solubility Limit	Low	The highest concentration (5120 ug/L) in the acute bioassay was above the solubility and the, second highest concentration (2560 ug/L) was near the limit. The solubility for this compound is 2700 ug/L. The solvent concentration used (4%) exceeded the recommended concentration of 1ml/l, and the percent survival in the control group was not reported.	
Domain 4: Test Organism				
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<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	The acute bioassays on adults were from in house cultures.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors report 5 replicates per treatment concentration and 10 randomly selected females per replicate.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 48 hr bioassay.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	The assessment within this acute bioassay appeared to be consistent among treatment and control groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to exposure influenced outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	The authors did not report how the dose-response curve was estimated with survival data.	
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text. Control data were not reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	Measures of variability in survival are reported in section 3.1 as standard error (SE).	
Additional Comments:	Survival data for controls were not reported. DEHP concentrations tested did not cause mortality. An LC 50 value could not be established.			
<b>Overall Quality Determination</b>		<b>Medium</b>		



<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Epigenetics-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical name was provided with correct nomenclature.	
	Metric 2: Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.	
	Metric 3: Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The controls are present and detailed in section 2.5 and in Figure 5.	
	Metric 5: Negative Control Response	High	Control gene expression after 24 days (6 days of chemical treatment and 18 days recovery) is presented in Figure 5.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and concentrations of the test substance were not measured during the study. For the 6 day exposure, the treatment solutions were not renewed; they were moved to new water without the chemical.	
	Metric 8: Consistency of Exposure Administration	High	This study only used one treatment concentration (0.1 ng/L), which was 10% of the 48hr LC50 for nauplii.	
	Metric 9: Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The study was for 24 days to allow for the zooplankton to produce multiple generations. The chemical exposure was for 6 days.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One treatment concentration (0.1 ng/L) was chosen to represent toxicity to 10% of the 48hr LC50 for nauplii.	
	Metric 12: Testing at or Below Solubility Limit	High	0.1 ng/L is under solubility limits. The Final Scope for DEHP lists the solubility for this compound as 2700 ug/L.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The gene expression experiment was conducted with inhouse copepod cultures.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.	
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<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Epigenetics-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	5 replicates per treatment group and per control group were used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 24 day study.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology assessed gene expression relative to two house-keeping genes.	
	Metric 18: Consistency of Outcome Assessment	High	Assessment within this gene expression experiment appeared to be consistent among treatment and control groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analysis was performed and described in section 2.6. Authors used z tests on bayesian z-scores to test for significance.	
	Metric 22: Reporting of Data	High	Section 3.4 and Figure 5 detail 6 day and 24 day gene expression for H3 and Hsp70. Data were represented as mean log2 (fold change) in expression (±SE; n=3) of Hsp70-like and H3 in treatments compared to the expression of the same genes in the control groups.	
	Metric 23: Explanation of Unexpected Outcomes	High	Measures of variability in gene expression relative to the control population are reported in section 3.4 as standard error (SE).	
Additional Comments:	H3 gene expression was downregulated after 6 days of DEHP exposure but not after 18 days of recovery. No changes in Hsp70-like expression were detected following DEHP exposure.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

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

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 24 day study.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology compared relative population size (+/- SE) to control replicate population size.
	Metric 18: Consistency of Outcome Assessment	High	Assessment within this multigenerational experiment appeared to be consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Authors used ANOVA and Tukey's post hoc to test for differences among treatment and control groups.
	Metric 22: Reporting of Data	High	Section 3.3 and Figure 4 detail 6 day exposure to DEHP effects on the population size (page 5/8). Relative <i>Parvocalanus crassirostris</i> population size (mean $\pm$ SE, n=4) was provided for each treatment group and control in Fig 4.
	Metric 23: Explanation of Unexpected Outcomes	High	Measures of variability in generation size relative to the control population are reported in section 3.3 as standard error (SE).

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

## Overall Quality Determination

**Medium**

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

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors report 10 replicates per treatment concentration and 20 females per replicate.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 5 day study.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology resulted in mean (+/- SE) for eggs/female for the control and for three treatment concentrations.
	Metric 18: Consistency of Outcome Assessment	High	The assessment within this reproduction experiment appeared to be consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Authors used ANOVA and Tukey's post hoc to test for differences among treatment and control groups.
	Metric 22: Reporting of Data	High	Section 3.2 details DEHP effects on the number of eggs per female, and Figure 3b presents the data for each treatment group and control (Page 5/8).
	Metric 23: Explanation of Unexpected Outcomes	High	Measures of variability in mean eggs per female are reported in section 3.2 as standard error (SE).

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

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Other (please specify below) (Population Size)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical name was provided with correct nomenclature.	
	Metric 2: Test Substance Source	Low	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard". Analytical verification was not reported by the author.	
	Metric 3: Test Substance Purity	High	The source was listed as Sigma Aldrich. The chemical grade was listed as "Pestanal analytical standard", from the Sigma-Aldrich page for this compound. The purity of this product is >98%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Controls are present and detailed in section 2.4 and in Figure 1.	
	Metric 5: Negative Control Response	High	Control population size after 24 days is presented in Figure 4. All treatment groups are compared as their % relative to the control population size at the end of the experiment.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and the concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	This study spanned over multiple generations (24 days) and only used one treatment concentration (0.11 ng/L), which was 10% of the 48hr LC50 for nauplii.	
	Metric 9: Measurement of Test Substance Concentration	Low	The study does not measure the compound and reports nominal treatment concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The study was 24 days to allow for the zooplankton to produce multiple generations.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	One treatment concentration (0.11 ng/L) was chosen to represent toxicity to 10% of the 48hr LC50 for nauplii.	
	Metric 12: Testing at or Below Solubility Limit	High	0.11 ng/L is under solubility limits. The Final Scope for DEHP lists the solubility for this compound as 2700 ug/L	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The multigeneration experiment was conducted with inhouse copepod cultures.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.	
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<b>Study Citation:</b>	Heindler, F. M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S. J., Vamvounis, G., Herwerden, van, L. (2017). Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, <i>Parvocalanus crassirostris</i> . <i>Ecotoxicology and Environmental Safety</i> 141:298-305.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Parvocalanus crassirostris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Other (please specify below) (Population Size)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3859142			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates per treatment group and control group were used. There were 36 adults and 68 nauplii per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Authors did not report environmental water quality conditions throughout the 24 day study.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported relative population size (+/- SE) compared to control replicates.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment within this multigenerational experiment appeared to be consistent among treatment and control groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported to indicate if they caused any differences.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was provided to suggest that health outcomes or other factors not related to the exposure influenced outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors used ANOVA and Tukey’s post hoc to test for differences among treatment and control groups.
	Metric 22:	Reporting of Data	High	Section 3.3 and Figure 4 detail 24 day exposure to DEHP effects on the population size (page 5/8). Relative <i>Parvocalanus crassirostris</i> population size (mean ± SE, n=4) were provided for each treatment group and control in Fig 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability in generation size relative to the control population are reported in section 3.3 as standard error (SE).
Additional Comments:	DEHP exposures for 6 and for 24 days significantly reduced the average population sizes (59±4.9% and 59±3.4% ) relative to the control.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	ADME (biotransformation)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	679685		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by chemical name and form. Radiolabeled DEHP was synthesized from carbonyl-labeled 1,2- dicarboxylic acid (o-phthalic acid) and 2-ethylhexanol and purified by thin-layer chromatography.
Metric 2:	Test Substance Source	Low	Radiolabeled DEHP was obtained from an individual, but the test substance identity was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	Uninformative	Study authors reported using a concurrent control group. But the control was an unoccupied exposure chamber (no test animal) that received 600 ppm diet (dosed with DEHP) at each feeding. This was more of an analytical control to ensure that the DEHP was primarily found in the feed (Table 2). A true negative control was not included in the experiment.
Metric 5:	Negative Control Response	Uninformative	There was no test animal placed in the control exposure chamber. Therefore, the biological response of the control could not be reported.
Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	Methods of dosing diets with radiolabeled DEHP were not provided, but the specific activities of the treatment groups at the beginning of the experiment were reported.
Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be administered consistently across study groups. It was reported that shrimp were fed at a rate of 1% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured. Specific activity of the feed was measured at the beginning and at the end of the experiment. Water samples were measured by scintillation counting at the beginning, prior to each feeding, and at the end of the experiment.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (24 and 96 hrs) was reported and appropriate for the study type.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
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<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.	
	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	1 organism per exposure chamber and 5 replicates per concentration were used.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Housing and environmental conditions during the 24 hr and 96 hr uptake studies were not reported.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcomes of interest. Shrimp samples were oxidized using a a Packard B306 tissue oxidizer and analyzed by scintillation counting.	
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was conducted after 24 and 96 hours of exposure.	
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21: Statistical Methods	High	ANOVA was performed to assess time and dose effects.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group (Table 3).	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	A true negative control was not included in the experiment. Methods of dosing diets with DEHP were not provided.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treatment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEHP. The control group, it seems, was fed the same diet, absent the test substance.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Methods of dosing diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not, or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in the feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from the nominal concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be administered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors were derived from measured concentrations (Fig 2).
	Metric 10:	Exposure Duration and Frequency	High	This was a 14 day dietary exposure to DEHP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study, which was to determine whether DEHP played a role in observed mortality rates in an aquaculture facility.
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<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in potential aqueous exposure.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted after 14 days of exposure.
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for the mortality data. There were negative findings, even at the highest concentration tested.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The methods of dosing diets with DEHP were not provided. Housing and environmental conditions during the 14 day test were not reported. Measured concentrations were different from nominal concentrations. There were negative findings for mortality, even in the highest concentration tested.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treatment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEHP. The control group, it seems, was fed the same diet, absent of the test substance.
	Metric 5:	Negative Control Response	Medium	The biological responses of the negative control group was reported, but there was DEHP contamination in the control (2 ppm vs 44-50,227 ppm in the treatment groups).
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The methods of dosing the diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from nominal concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported and exposures appear to be administered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors derived from the measured concentrations (Fig 2).
	Metric 10:	Exposure Duration and Frequency	High	This was a 14 day dietary exposure to DEHP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
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<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in aqueous exposure.
	Metric 17:	Outcome Assessment Methodology	Medium	Detailed methods for determining DEHP body burden were not provided.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted after 14 days of exposure.
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report measures of variability for whole-body residues of DEHP for all treatment groups.
Additional Comments:	Methods of preparing diets with DEHP were not provided. Housing and environmental conditions during the 14 day test were not reported. Measured concentrations were different from nominal concentrations. There was DEHP contamination in the control group. The study did not report measures of variability for whole-body residues of DEHP for all treatment groups.			

## Overall Quality Determination

Low

<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance is identified by the accepted name [1,2-di-2-ethylhexyl phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. Treatment groups received feed equivalent to 4% bodyweight over two feedings per day, with feed containing the appropriate dose of DEHP. The control group, it seems, was fed the same diet, absent of the test substance.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The methods of dosing diets with DEHP were not provided. It was not reported whether a solvent carrier was used or not, or whether DEHP dosed diets were prepared daily and were homogenized well. The concentration of DEHP in the feed was provided, but it was not clear whether these measurements were taken at the beginning or at the end of the 14 day experiment. The measured concentrations deviated from nominal concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Details of the exposure administration were reported, and exposures appear to be administered consistently across study groups. It was reported that shrimp were fed at a rate of 4% body weight per day in 2 equal feedings. The steps taken to ensure uniform feeding/exposure for all test organisms were not provided.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but it was unclear whether the measurements were taken at the beginning or at the end of the experiment. Measured concentrations were different from nominal concentrations. Bioaccumulation factors were derived from the measured concentrations (Fig 2).
	Metric 10:	Exposure Duration and Frequency	High	This was a 14 day dietary exposure to DEHP.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The source was reported, but details of the test organism characteristics were lacking.
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<b>Study Citation:</b>	Hobson, J. F., Carter, D. E., Lightner, D. V. (1984). Toxicity of a phthalate ester in the diet of a penaeid shrimp. Journal of Toxicology and Environmental Health 13(4-6):959-968.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (marine); Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaeus vannamei</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679685			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Six organisms per test vessel and 3 to 4 replicates per concentration (Table 1) were used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing and environmental conditions during the 14 day study were not reported. It was not reported whether unused feed was removed after each feeding, which could otherwise result in potential aqueous exposure.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted after 14 days of exposure.
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Statistical analysis was not necessary for development/growth data (molting).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were presented in the results section in the text as a general assessment.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The methods of dosing diets with DEHP were not provided. Housing and environmental conditions during the 14 day test were not reported. Measured concentrations were different from nominal concentrations.			
Overall Quality Determination		Medium		



<b>Study Citation:</b>	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on <i>Karenia brevis</i> . Chemosphere 155:498-508.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Karenia brevis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3230225			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Relevant test materials are described as di-iso-butyl ortho-phthalate (DIBP), benzyl-n-butyl ortho-phthalate (BBP), di-n-butylortho-phthalate (DBP), and bis(2-ethylhexyl)ortho-phthalate(DEHP). No further details are provided.
	Metric 2:	Test Substance Source	Low	Chemicals are sourced by the Sigma Company; no additional information is provided.
	Metric 3:	Test Substance Purity	High	The purity is reported as > 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative and solvent controls were used. The authors indicated that no significant differences were observed between the solvent control (acetone concentration (0.5 mL L-1)) and the negative control.
	Metric 5:	Negative Control Response	High	No adverse effects were reported in the control.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and minimize loss of the test substance before and during the exposure for these degradable substances. A solvent (acetone) was used to facilitate the preparation 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 chemicals meant that exposure concentrations may have differed because of degradation, but this was not discussed.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported. As degradation was likely to be observed, the reported nominal concentrations were not likely to be representative of the final concentration, and reporting in terms of nominal concentrations may have underestimated the effects observed.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (96-hour algae growth inhibition test).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Exposure concentrations were reported to be 0, 1, 5, 10, 20, 30, 50, 100, 150, 200 mL/L. These concentrations of DEHP did not inhibit algal growth.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate (the authors demonstrated that no significant difference (p > 0.05) was observed between the growth in controls and acetone treatment.).
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<b>Study Citation:</b>	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on <i>Karenia brevis</i> . Chemosphere 155:498-508.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Karenia brevis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3230225			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	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 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 algal density) were not reported in each treatment group and control.	
Domain 5: Outcome Assessment				
	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 ± 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 no significant effects were observed on growth following exposure to DEHP, but no data were provided.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistent across groups. Cell numbers were determined every 24 hours.	
Domain 6: Confounding / Variable 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 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 Presentation and Analysis				
	Metric 21: Statistical Methods	Low	One-way ANOVA was adopted to determine the significant differences between experimental and control groups. The calculations and measures of significance were not provided, so no conclusions about a dose response could be made.	
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.	
Additional Comments:	There was no significant effect on algal growth following DEHP exposure. The discussion of growth inhibition following exposure to DEHP, DIBP, BBP and DBP was lacking.			
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<b>Study Citation:</b>	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on <i>Karenia brevis</i> . Chemosphere 155:498-508.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	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)
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Karenia brevis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3230225

Domain	Metric	Rating	Comments
<b>Overall Quality Determination</b>		<b>Low</b>	

<b>Study Citation:</b>	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. <i>Ecotoxicology and Environmental Safety</i> 5(2):202-210.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were reported.
	Metric 2:	Test Substance Source	High	The test substance source includes: Aldrich Chemical Company for the unlabeled phthalate 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 negative controls were reported.
	Metric 5:	Negative Control Response	N/A	No negative controls were reported.
	Metric 6:	Randomized Allocation	Medium	Organisms were collected from Galveston Bay. The allocation method to exposure groups was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for the test preparation was described. Measures taken to account for P-chem properties were not reported.
	Metric 8:	Consistency of Exposure Administration	High	No variations in exposure administration were reported.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using analytical techniques, gas-liquid chromatography and liquid scintillation
	Metric 10:	Exposure Duration and Frequency	Medium	There was a 24-hour exposure period.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Only two exposure groups were reported.
	Metric 12:	Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Organisms characteristics were not described in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were acclimatized for 4 days prior to phthalate exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was a lower than typical number of organisms in the exposure groups.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was addressed.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was consistent for all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were indicated in the assessment.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Presentation and Analysis				
	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	No unexpected outcomes were reported.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>sheepshead minnow</i> ( <i>Cyprinodon variegatus</i> ); Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316224; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test material was identified, and the CASRN was given.	
	Metric 2: Test Substance Source	High	The source was listed as EG&G Bionomics Aquatic Toxicology Laboratory in Wareham, Massachusetts. No other information about the source was given.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used.	
	Metric 5: Negative Control Response	High	No mortality was reported in the controls.	
	Metric 6: Randomized Allocation	Medium	Test organisms were impartially distributed to each chamber (pdf pg 136).	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and the methods for preparation of the test media were described in adequate detail and accounted for the properties of the test material. For all low-solubility phthalates, an enhanced mixing procedure was used, while for the butyl benzyl phthalate exposure, microbial degradation was accounted for with a cleaning 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 the exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Concentrations were measured using GS-MS to account for poor water solubility.	
	Metric 10: Exposure Duration and Frequency	High	A 96 hour exposure was appropriate for an acute test.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Test concentration spacing was limited, as this was designed as a limit test up to the solubility limit of the chemical.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit, which were reported in Appendix A (pdf pg 164).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Specimens were either cultured at the Laboratory or purchased commercially. All fish were tested as juveniles, <10weeks old.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	A 96 hour acclimation period was reported.	

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<b>Study Citation:</b>	Bionomics,, Springborn (1984). Acute toxicity of thirteen phthalate esters to the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) (final report).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>sheepshead minnow (Cyprinodon variegatus)</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316224; Linked HERO ID(s): 1321996, 1316224			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	Only two replicates of 10 fish were used in each treatment.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of health, and biomass loading was appropriate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes(s) of interest (mortality).	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	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 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:	The 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, butyl benzyl phthalate was referred to as 1D, DIDP was referred to as 1L, and DINP was referred to as 1J.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Sugawara, N. (1974). Toxic effect of a normal series of phthalate esters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-89.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Artemia salina</i> ; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1315792			
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 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	Medium	Hatch rate of ~47% in the control seems low.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The study provided only limited details on the measures taken to appropriately prepare test concentrations, Triton X-100 used as a carrier. The experimental set up and the type of experimental vessel used were not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	A one-time dose was used. The volume of test solution in experimental dishes was not reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	The text states 40 or 72 hours was the exposure duration. Figure 2 shows the number of eggs hatched after 40 hours. DOP is shown in this figure.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Three treatment levels with adequate spacing were used.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Since the phthalates used in this study were not completely soluble, 20uL of Triton X-100 was added and vigorously shaken. The solvent concentration seemed high. "The concentration of Triton X-100 in the control, 10 ppm, and 20-ppm solutions was adjusted to 10 ppm by adding this reagent."	
Domain 4: Test Organism				
	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 to 2 mg of eggs were placed in the dishes (seems like a wide range and no replicates were reported).	
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<b>Study Citation:</b>	Sugawara, N. (1974). Toxic effect of a normal series of phthalate esters on the hatching of shrimp eggs. Toxicology and Applied Pharmacology 30(1):87-89.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Artemia salina</i> ; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1315792		
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	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 limited.
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	High	Statistical analysis was performed. The Fig 1 caption states that student's t test was used.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group in Figure 2. The legend for the graph is not included. A general overview of the results was described in the text, but not many details were provided.
	Metric 23: Explanation of Unexpected Outcomes	Low	Insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.
Additional Comments:	This evaluation is for mortality assessment after exposure to DOP. DOP is the same chemical compound as DEHP, hence why DEHP was chosen for the chemical inventory form. Mortality of shrimp eggs in the control was ~47%.		
<b>Overall Quality Determination</b>		<b>Low</b>	

<b>Study Citation:</b>	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 sheephead minnows. <i>Ecotoxicology and Environmental Safety</i> 5(2):202-210.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Crassostrea virginica</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.
	Metric 2:	Test Substance Source	High	The test substance source includes: Aldrich Chemical Company for the unlabeled phthalate and California Bionuclear Corporation for the 14C-labeled phthalate.
	Metric 3:	Test Substance Purity	Low	The purity of the chemical was not included in the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	An experiment with control to test the absorbance of empty oyster shells was conducted. However, no controls were used for the experiment that measured concentrations in organism tissue samples.
	Metric 5:	Negative Control Response	N/A	No negative controls were reported.
	Metric 6:	Randomized Allocation	Low	Organisms were collected from Galveston Bay. The allocation method to the exposure groups was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for the test preparation was described. Measures taken to account for P-chem properties were not reported.
	Metric 8:	Consistency of Exposure Administration	High	No variations in the exposure administration were reported.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using analytical techniques- gas-liquid chromatography and liquid scintillation
	Metric 10:	Exposure Duration and Frequency	Medium	This was a 24-hour exposure period.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Only two exposure groups were reported.
	Metric 12:	Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Organism characteristics were not described in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were acclimatized for 4 days prior to phthalate exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	A lower than is typical number of organisms was used in the exposure groups.
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Mollusks; <i>Crassostrea virginica</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology was addressed.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent for all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were indicated in the assessment.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A three-way analysis of variance (ANOVA) on the data was performed using the General Linear Model procedure of SAS 76.
	Metric 22:	Reporting of Data	High	Data for each outcome was reported.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	The concentrations during the 24-hr exposure period remained relatively constant with the exception of the oyster experiments. The concentrations decreased 30 to 70% during the oyster exposures, probably due to the high adsorptive capacity of the shells. The assertion was supported by an experiment showing absorption of chemical concentration in empty oyster shells.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [di(ethyl-hexyl)-phthalate, DEHP].
	Metric 2:	Test Substance Source	Low	The only test substance source information that was given was the manufacturer."All test substances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.Certain test substances required DMSO to be used as a carrier solvent at a maximum concentration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used the carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately reported for each individual chemical tested.The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were therefore an overestimation of actual contaminants present."
	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 (nominal concentrations used).
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.
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<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.	
	Metric 22: Reporting of Data	Medium	Endpoints were reported but treatment values were not.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This evaluation is for the 96H LC50 value.			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [di(ethyl-hexyl)-phthalate, DEHP].
	Metric 2:	Test Substance Source	Low	The only test substance source information that was given was the manufacturer."All test substances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.Certain test substances required DMSO to be used as a carrier solvent at a maximum concentration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately reported for each individual chemical tested.The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were therefore an overestimation of actual contaminants present."
	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 (nominal concentrations used).
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.
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<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.	
	Metric 22: Reporting of Data	Medium	Endpoints were reported but treatment values were not.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This evaluation is for the 10 d LOEC and NOEC values.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by the accepted name [di(ethyl-hexyl)-phthalate, DEHP].
	Metric 2:	Test Substance Source	Low	The only test substance source information given is the manufacturer."All test substances were purchased from Sigma (St. Louis, MO)."The test substance identity was NOT analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.Certain test substances required DMSO to be used as a carrier solvent at a maximum concentration of 0.1 mL/L, and for those test substances, a "carrier solvent control group of six replicates with the highest concentration of DMSO used in the test" was used. However, the study does not state which of the test substances used the carrier solvent and which did not.
	Metric 5:	Negative Control Response	High	The biological responses were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were not adequately reported for each individual chemical test.The test containers were covered with a glass lid, and this was the only measure taken to maintain the test substance concentration. The study authors note "[our] results referred to nominal concentrations and were therefore an overestimation of actual contaminants present."
	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 (nominal concentrations used).
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure was less than 28 days but probably close to 14 days based on graph depiction.
	Metric 11:	Number of Exposure Groups/ 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. DMSO was used to aid solubility for certain test substances, but it was not explicitly reported if DMSO was used for the DEHP groups.
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<b>Study Citation:</b>	Forget-Leray, J., Landriau, I., Minier, C., Leboulenger, F. (2005). Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod <i>Eurytemora affinis</i> (Poppe). <i>Ecotoxicology and Environmental Safety</i> 60(3):288-294.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	679508			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The test organisms were adequately described but were wild caught.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism environmental conditions were conducive to the maintenance of health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical methods were described.	
	Metric 22: Reporting of Data	High	Developmental time was reported via graph.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This evaluation is for the inhibition of larval development.			
Overall Quality Determination		High		

<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316220			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by chemical name. No CASRN or structure were provided.
	Metric 2:	Test Substance Source	High	The source of the phthalates was Bionomics Aquatic Toxicology Laboratory (Wareham, MA).
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the substance were not included in the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors reported using negative controls.
	Metric 5:	Negative Control Response	High	The response of the negative controls was adequate.
	Metric 6:	Randomized Allocation	Medium	Mysid shrimp were maintained 1-3 days before they were distributed into test vessels.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental design followed protocol guidelines.
	Metric 8:	Consistency of Exposure Administration	High	Authors reported consistent administration.
	Metric 9:	Measurement of Test Substance Concentration	High	Phthalates were analytically verified and measured.
	Metric 10:	Exposure Duration and Frequency	High	The test duration followed protocol.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	This study only performed a single exposure (0.44 mg/L), as a range finding test reported no effect at levels below the water solubility limit.
	Metric 12:	Testing at or Below Solubility Limit	Low	The single concentration used for DEHP was 0.44 mg/L and is over the solubility listed in the Final Scope (0.27 mg/L).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The source of the organisms was reported. Details beyond that were not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were housed for 1-3 days prior to treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Replicates followed protocol.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate and described in detail.
	Metric 17:	Outcome Assessment Methodology	High	Outcomes were reported and addressed.

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<b>Study Citation:</b>	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to mysid shrimp (Mysidopsis bahia).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Mysidopsis bahia</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1316220			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed and reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No differences were reported.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	No data analysis was presented as the LC50 was >0.44 mg/L.	
	Metric 22: Reporting of Data	High	Data was reported adequately.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
Additional Comments:	Based on preliminary studies, concentrations of DEHP below the solubility limit did not result in adverse outcomes; therefore, a single concentration was included in the final study.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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 ( <i>Alburnus alburnus</i> ) and the harpacticoid <i>Nitocra spinipes</i> . Chemosphere 8(11-12):843-851.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Nitocra spinipes</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	51937			
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	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Only general methods of exposure administration were reported, so an assessment of the exposure methods was difficult.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure and the exposure frequency were reported and suitable, but they were slightly longer than typical for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	At least 6 concentrations were tested, but a range was not reported.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether the exposure concentrations exceeded the water solubility limit. However, the given LC50 (>300 mg/L) is well above the water solubility given in the Final Scope for DEHP, 0.27 mg/L at 25C. This suggests that no toxicity was observed at saturation.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was unclear if the test organisms were acclimatized to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 20 organisms with no replicates per treatment used.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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 ( <i>Alburnus alburnus</i> ) and the harpacticoid <i>Nitocra spinipes</i> . Chemosphere 8(11-12):843-851.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Nitocra spinipes</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	51937			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and seemed consistent.
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.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the table.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The given LC50 is unbounded (>300 mg/L), which is well above the water solubility limit given in the Final Scope for DEHP (0.27 mg/L at 25C), which suggests that no toxicity was observed at saturation.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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. <i>Ecotoxicology and Environmental Safety</i> 5(2):202-210.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaecus aztecus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name. No CASRN or structure were provided.
	Metric 2:	Test Substance Source	High	Test substance sources include Aldrich Chemical Company for the unlabeled phthalate and California Bionuclear Corporation for the 14C-labeled phthalate.
	Metric 3:	Test Substance Purity	Low	
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	Negative controls were not reported in the study.
	Metric 5:	Negative Control Response	N/A	Negative controls were not reported in the study.
	Metric 6:	Randomized Allocation	Medium	Organisms were collected from Galveston Bay. The allocation method to the exposure groups was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental design for the test preparation was described.
	Metric 8:	Consistency of Exposure Administration	High	No variations in exposure administration were reported.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using analytical techniques including gas-liquid chromatography and liquid scintillation.
	Metric 10:	Exposure Duration and Frequency	Medium	This was a 24-hour exposure period.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Only two exposure groups were reported.
	Metric 12:	Testing at or Below Solubility Limit	High	There were 2 concentrations below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Characteristics were not described in the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms were acclimatized for 4 days prior to phthalate exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was a lower than typical number of organisms in the exposure groups.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Penaecus aztecus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789995			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	High	The methodology was addressed.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was consistent for all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were indicated in the assessment.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to the exposure were not reported.
Domain 7: Data Presentation and Analysis				
	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	
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	None			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. PLoS ONE 10(7):e0130896.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Bos taurus</i> , <i>Holstein Fresian</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3071101

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The test conditions were adequate for the test species.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology measured the intended outcomes of interest (in vitro fertilization and embryo maturation).
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the control and exposure groups outside the test substance administration.
	Metric 20: Outcomes Unrelated to Exposure	High	The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Data were arcsine-transformed prior to one-way ANOVA followed by the Tukey-Kramer test.
	Metric 22: Reporting of Data	High	Data on developmental effects was presented in Figures 7-9.
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in oocyte developmental effects and embryo competence from test substance exposures.
Additional Comments: None			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. PLoS ONE 10(7):e0130896.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Bos taurus</i> , <i>Holstein Fresian</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071101			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl) phthalate (DEHP) and a commercial name, Oxoplast O 30, along with purity and impurity.	
Metric 2:	Test Substance Source	High	The source was identified as ZAK Spolka Akcyjna, a major chemical manufacturer in Poland.	
Metric 3:	Test Substance Purity	High	The test substance purity was 99.5%, with impurities less than or equal to 0.1%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Negative controls (N = 5) were tube-fed with water.	
Metric 5:	Negative Control Response	High	Controls responded as expected.	
Metric 6:	Randomized Allocation	Low	The researchers did not report randomization.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was adequately detailed.	
Metric 8:	Consistency of Exposure Administration	High	Test substance exposures were administered consistently across the control and study group.	
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations (100 mg/kg of body weight) were measured prior to administration via tube-feeding.	
Metric 10:	Exposure Duration and Frequency	High	The exposure duration (3 days) and frequency (daily) were appropriate for the outcomes measured.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Single exposure concentration versus the control may be appropriate for determining effects for the purpose of the study, but would not be useful for determining a dose-response relationship.	
Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was administered via tube-feeding.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Medium	The study was conducted with female test species on an experimental dairy farm, but no other details (i.e. age, weight/size, etc.) were provided.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were adequate and consistent across the control and exposed test groups.	
Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of controls (N = 5) and exposure test organisms (N = 4) were adequate for characterizing toxicological effects.	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	High	The test conditions were adequate for the test species.	
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<b>Study Citation:</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. PLoS ONE 10(7):e0130896.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Bos taurus</i> , <i>Holstein Friesian</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071101			
Domain	Metric	Rating	Comments	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology measured the intended outcomes of interest (follicular and corpus luteum development and hormone concentrations).	
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the control and exposure groups outside the test substance administration.	
	Metric 20: Outcomes Unrelated to Exposure	High	The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Data were analyzed by one-way ANOVA followed by the Student's t-test.	
	Metric 22: Reporting of Data	High	Data on developmental effects was presented in Figures 3-6.	
	Metric 23: Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in developmental effects from test substance exposures.	
Additional Comments: None				
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. PLoS ONE 10(7):e0130896.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Bos taurus</i> , <i>Holstein Fresian</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Endocrine toxicity-Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071101			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as di(2-ethylhexyl) phthalate (DEHP) and a commercial name, Oxoplast O 30, along with purity and impurity.
	Metric 2:	Test Substance Source	High	The source was identified as ZAK Spolka Akcyjna, a major chemical manufacturer in Poland.
	Metric 3:	Test Substance Purity	High	The test substance purity was 99.5%, with impurities less than or equal to 0.1%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls (N = 5) were tube-fed with water.
	Metric 5:	Negative Control Response	High	Controls responded as expected.
	Metric 6:	Randomized Allocation	Low	The researchers did not report randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was adequately detailed.
	Metric 8:	Consistency of Exposure Administration	High	Test substance exposures were administered consistently across the control and study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations (100 mg/kg of body weight) were measured prior to administration via tube-feeding. Metabolites were measured from blood, urine and follicular fluid, and milk from both control and treated test organisms.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration (3 days) and frequency (daily) were appropriate for the outcomes measured.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Single exposure concentration versus the control may be appropriate for determining effects for the purpose of the study, but would not be useful for determining a dose-response relationship.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was administered via tube-feeding.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The study was conducted with female test species on an experimental dairy farm, but no other details (i.e. age, weight/size, etc.) were provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were adequate and consistent across the control and exposed test groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of controls (N = 5) and exposure test organisms (N = 4) were adequate for characterizing toxicological effects.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Kalo, D., Hadas, R., Furman, O., Ben-Ari, J., Maor, Y., Patterson, D. G., Tomey, C., Roth, Z. (2015). Carryover effects of acute DEHP exposure on ovarian function and oocyte developmental competence in lactating cows. PLoS ONE 10(7):e0130896.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Bos taurus</i> , <i>Holstein Fresian</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Endocrine toxicity-Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3071101			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were adequate for the test species.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology measured the intended outcomes of interest (metabolites of DEHP).
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was sufficiently detailed and consistently applied across the control and exposure groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the control and exposure groups outside the test substance administration.
	Metric 20:	Outcomes Unrelated to Exposure	High	The study authors did not indicate outcomes unrelated to exposures or differences among the study groups that would affect the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	DEHP-metabolite mean concentrations were analyzed by one-way ANOVA followed by Student's t test.
	Metric 22:	Reporting of Data	High	Data on developmental effects was presented in Table 2 and Figure 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors adequately discussed interspecies differences in DEHP metabolite concentrations from test substance exposures.
Additional Comments:	This form is to account for cellular and biochemical outcomes of follicle development in the ovaries and the effect on progesterone in the plasma.			

**Overall Quality Determination****High**

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

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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	20 males and 20 females were selected and divided into five groups equal in size. The use of replication was unclearly described.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for marmosets was maintained at a room temperature of 26 ± 2°C, a relative humidity of 50 ± 10%, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and additives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given as supplemental food. Tap water passed through a 5-µm filter and irradiated by ultraviolet was freely available to the animals from water bottles.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding / 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. There are no limitations that would result in a substantial impact on results.	
	Metric 20: Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced disproportionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an influence on the mean values of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathological changes suggestive of tract impairment were noted in those animals.	
Domain 7: Data Presentation and Analysis				

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

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Endocrine			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical is identified as di(2-ethylhexyl)phthalate (DEHP), but no other identifying information is provided.
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	DEHP was dissolved in corn oil and administered orally with a catheter once daily in the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.
	Metric 8:	Consistency of Exposure Administration	High	The exposure solution volume or the number of molecules of the test substance per container were the same across replicates and groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Authors allude to verification of the test material concentration, but exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Dosages of 2500, 500, and 100 mg/kg were chosen based on the results of range-finding tests conducted before the initiation of the definitive test.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was dietary exposure, so solubility was not relevant as it was administered via gavage.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organisms were sourced from "CLEA JAPAN" and were separated by sex. Age of the test organisms was not specified, which could have affected the outcome of the test.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Marmosets were acclimated for 3 months in the same conditions as the test.
	Metric 15:	Number of Organisms and Replicates per Group	Low	20 males and 20 females were selected and divided into five groups equal in size. The use of replication was unclearly described.
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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult		
<b>Health Outcome:</b>	Endocrine		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	630680		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for marmosets was maintained at a room temperature of $26 \pm 2^{\circ}\text{C}$ , a relative humidity of $50 \pm 10\%$ , a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and additives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given as supplemental food. Tap water passed through a 5- $\mu\text{m}$ filter and irradiated by ultraviolet was freely available to the animals from water bottles.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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. There are no limitations that would result in a substantial impact on results.
Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced disproportionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an influence on the mean values of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathological changes suggestive of tract impairment were noted in those animals.
Domain 7: Data Presentation and Analysis			
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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Endocrine			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group variances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.
Additional Comments:	The authors reported effect on endocrine activity by characterizing the effects on the levels of blood testosterone, estradiol, and cholecystokinin in the test organisms. They also examine organ weight of the testis			

**Overall Quality Determination****Medium**

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

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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Other (please specify below) (Organ Weight)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for marmosets was maintained at a room temperature of 26 ± 2°C, a relative humidity of 50 ± 10%, a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and additives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given as supplemental food. Tap water passed through a 5-µm filter and irradiated by ultraviolet was freely available to the animals from water bottles.	
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.	
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	
Domain 6: Confounding / 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. There are no limitations that would result in a substantial impact on results.	
Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced disproportionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an influence on the mean values of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathological changes suggestive of tract impairment were noted in those animals.	
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult
<b>Health Outcome:</b>	Other (please specify below) (Organ Weight)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	630680

Domain	Metric	Rating	Comments
	Metric 21: Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group variances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.

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

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical is identified as di(2-ethylhexyl)phthalate (DEHP), but no other identifying information is provided.
	Metric 2:	Test Substance Source	Low	The chemical was purchased from Tokyo Chemical Co. Ltd. No other information was provided.
	Metric 3:	Test Substance Purity	High	The chemical purity was 99.3%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group was used and referred to in all tables and text, but no additional details were provided.
	Metric 5:	Negative Control Response	High	The biological responses (e.g., weight) of the negative control group(s) were adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	DEHP was dissolved in corn oil and administered orally with a catheter once daily in the morning for 13 weeks (91 days consecutively) with a volume of 5 ml/kg. This was appropriate given the high LogKOW of the chemical.
	Metric 8:	Consistency of Exposure Administration	High	The exposure solution volume or the number of molecules of the test substance per container were the same across replicates and groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Authors allude to verification of the test material concentration, but exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	Low	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (daily gavage via oral catheter).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Dosages of 2500, 500, and 100 mg/kg were chosen based on the results of range-finding tests conducted before the initiation of the definitive test.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This was a dietary exposure, so the solubility was not relevant as it was administered via gavage.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organisms were sourced from "CLEA JAPAN" and were separated by sex. Age of the test organisms was not specified, which could have affected the outcome of the test.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Marmosets were acclimated for 3 months in the same conditions as the test.
	Metric 15:	Number of Organisms and Replicates per Group	Low	20 males and 20 females were selected and divided into five groups equal in size. The use of replication was unclearly described.
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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	630680		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	High	Throughout the study including acclimatization, the exclusive rearing room for marmosets was maintained at a room temperature of $26 \pm 2^{\circ}\text{C}$ , a relative humidity of $50 \pm 10\%$ , a ventilation of 15 times per hour with fresh filtered air, and a lighting period of 12 h (from 8:00 to 20:00). Animals were individually housed in a stainless steel wire-mesh cage (400 w X 600 d X 650 h mm) during the acclimatizing and treatment periods. The mixture of pelleted diet for New World monkeys (CMS-1, CLEA JAPAN Inc.) and additives (water and ascorbic acid), prepared every day as a main diet, was given to each animal in the morning during the acclimatizing period and after administration during the experimental period. Boiled eggs and bananas were also given as supplemental food. Tap water passed through a 5- $\mu\text{m}$ filter and irradiated by ultraviolet was freely available to the animals from water bottles.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest, and the assessment methodology was sensitive and appropriate for the outcomes(s) of interest.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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. There are no limitations that would result in a substantial impact on results.
Metric 20:	Outcomes Unrelated to Exposure	Low	Reported information indicated that one or more study groups experienced disproportionate test organism attrition or outcomes unrelated to exposure. Males were reported to have large spleens, and diarrhea and bloody or tarry stools were occasionally observed in some specific females of the 100 and 500 mg/kg DEHP and clofibrate groups. These changes were accompanied by decrease in body weight in some animals, having an influence on the mean values of the 500 mg/kg DEHP and the clofibrate groups. However, they were all transient findings, and no histopathological changes suggestive of tract impairment were noted in those animals.
Domain 7: Data Presentation and Analysis			
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<b>Study Citation:</b>	Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M., Katoh, M. (1998). Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: Lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 42(1):49-56.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Common marmosets (Callithrix jacchus)</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	630680			
Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	All groups were compared by analysis of variance (ANOVA), and Dunnett's multiple range test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group) was used when intergroup differences were found to be significant. When the results of Bartlett's test indicated heterogeneous group variances, all groups were compared by the Kruskal-Wallis test and Dunnett's rank sum test (for an equal number of animals in each group) or Scheffe's test (for unequal numbers of animals in each group).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.
Additional Comments:	The authors reported the effect on growth by reporting the weight differences between the control and treatment groups after 13 weeks. As the age of the test organisms was not provided, it was unclear whether this was an effect on growth or weight loss.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Wang, H., Guan, T. Q., Sun, J. X., Talukder, M., Huang, Y. Q., Li, Y. H., Li, J. L. (2020). Di-(2-ethylhexyl) phthalate induced nephrotoxicity in quail ( <i>Coturnix japonica</i> ) by triggering nuclear xenobiotic receptors and modulating the cytochrome P450 system. <i>Environmental Pollution</i> 261:114162.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Coturnix japonica</i> ; Juvenile			
<b>Health Outcome:</b>	Renal/Kidney			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	6816734			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	CAS, formula, and source are all listed for DEHP. This info is in the supplementary information document. The purity was >99%.	
Metric 2:	Test Substance Source	High	The source and purity from the manufacturer were reported. This info is in the supplementary information document. The source was reported to be Aladdin Biochemical Technology Co., (Shanghai, China).	
Metric 3:	Test Substance Purity	High	The chemical manufacturer reported the purity at >99%. This info is in the supplementary information document.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	There is a control group with water and a vehicle control group with corn oil as a negative control. This info is in the supplementary information document.	
Metric 5:	Negative Control Response	High	The responses for histology assessment scores are the same (totals are 0). This information is in Table S2 in the Supplemental S1 materials. This was a histopathological assessment.	
Metric 6:	Randomized Allocation	Low	Random allocation to treatment groups was not reported.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	The test concentrations were not verified analytically.	
Metric 8:	Consistency of Exposure Administration	Medium	Treatments appear to be administered consistently; however, details on the protocol are not well described.	
Metric 9:	Measurement of Test Substance Concentration	Low	This study did not verify DEHP concentrations in the treatment groups.	
Metric 10:	Exposure Duration and Frequency	High	The study was conducted daily for over 45 days.	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	The treatment concentrations and control groups represent a wide range of high DEHP concentrations for this study. The study provides no details on the numbers of individuals in each group, so there is no way to know if they are represented equally.	
Metric 12:	Testing at or Below Solubility Limit	N/A	This is a gavage study, so solubility is not a factor in the treatment.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	The supplementary submission for this paper adequately described the test organisms.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	An acclimatization period was not described.	

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<b>Study Citation:</b>	Wang, H., Guan, T. Q., Sun, J. X., Talukder, M., Huang, Y. Q., Li, Y. H., Li, J. L. (2020). Di-(2-ethylhexyl) phthalate induced nephrotoxicity in quail ( <i>Coturnix japonica</i> ) by triggering nuclear xenobiotic receptors and modulating the cytochrome P450 system. <i>Environmental Pollution</i> 261:114162.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Coturnix japonica</i> ; Juvenile			
<b>Health Outcome:</b>	Renal/Kidney			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	6816734			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	These details were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The methods do not indicate that there are differences among treatment groups.	
	Metric 17: Outcome Assessment Methodology	High	The doses were specifically selected to produce organ level impacts and not mortality. The endpoints are representative for this chronic study.	
	Metric 18: Consistency of Outcome Assessment	Medium	The assessment of outcomes appears consistent; however, the sample sizes are not reported.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There appears to be no confounding variables for this study.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no indication that the differences were attributed to unrelated health outcomes.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	The histological review of kidney tissue was qualitative and no stats were performed.	
	Metric 22: Reporting of Data	Low	A "semiquantitative" series of scores for histology observations were reported. No details were provided on the methods and number of people scoring these observations.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	No unexplained outcomes were reported.	
Additional Comments:	This review is on the kidney histology performed for this paper.			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Wang, H., Guan, T. Q., Sun, J. X., Talukder, M., Huang, Y. Q., Li, Y. H., Li, J. L. (2020). Di-(2-ethylhexyl) phthalate induced nephrotoxicity in quail ( <i>Coturnix japonica</i> ) by triggering nuclear xenobiotic receptors and modulating the cytochrome P450 system. <i>Environmental Pollution</i> 261:114162.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Coturnix japonica</i> ; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	6816734			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	CAS, formula, and source are all listed for DEHP. This info is in the supplementary information document.	
Metric 2:	Test Substance Source	High	The source and purity from the manufacturer were reported. This info is in the supplementary information document. The source was reported to be Aladdin Biochemical Technology Co., (Shanghai, China).	
Metric 3:	Test Substance Purity	High	The chemical manufacturer reported the purity at >99%. This info is in the supplementary information document.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	There is a control group with water and a vehicle control group with corn oil as a negative control. This info is in the supplementary information document.	
Metric 5:	Negative Control Response	High	The responses for mechanistic endpoints are the same between control and vehicle control.	
Metric 6:	Randomized Allocation	Low	Random allocation to treatment groups was not reported.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	The test concentrations of the feeds were not verified analytically. The formulation for these treatments was not well described.	
Metric 8:	Consistency of Exposure Administration	Medium	Treatments appear to be administered consistently; however, details on treatment protocol are not well described.	
Metric 9:	Measurement of Test Substance Concentration	Low	This study did not verify DEHP concentrations in the treatment groups.	
Metric 10:	Exposure Duration and Frequency	Medium	The treatments were administered daily for 45 days. The frequency of administration per day was unclear.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The treatment concentrations and control groups represent a wide range of high DEHP concentrations for this study. The study provides no details on the numbers of individuals in each group, so there is no way to know if they are represented equally.	
Metric 12:	Testing at or Below Solubility Limit	N/A	This is a gavage treatment administration, so solubility is not a factor in the treatment.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	The supplementary submission for this paper adequately described the test organisms.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	An acclimatization period was not described.	
Metric 15:	Number of Organisms and Replicates per Group	Low	These details were not reported.	
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<b>Study Citation:</b>	Wang, H., Guan, T. Q., Sun, J. X., Talukder, M., Huang, Y. Q., Li, Y. H., Li, J. L. (2020). Di-(2-ethylhexyl) phthalate induced nephrotoxicity in quail ( <i>Coturnix japonica</i> ) by triggering nuclear xenobiotic receptors and modulating the cytochrome P450 system. Environmental Pollution 261:114162.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Coturnix japonica</i> ; Juvenile
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	6816734

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The methods do not indicate that there are differences among treatment groups.
	Metric 17: Outcome Assessment Methodology	High	The doses were specifically selected to produce organ level impacts and not mortality. The endpoints are representative for this chronic study.
	Metric 18: Consistency of Outcome Assessment	Medium	The assessment of outcomes appears consistent; however, the sample sizes are not reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There appears to be no confounding variables for this study.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no indication that the differences were attributed to unrelated health outcomes.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The statistics for molecular and CypP450 endpoints are described.
	Metric 22: Reporting of Data	High	The statistical methods for the mechanistic endpoints are well described in the main paper and the supplementary information.
	Metric 23: Explanation of Unexpected Outcomes	Medium	No unexplained outcomes were reported.
Additional Comments: This review is on the mechanistic endpoints (Cyp450 enzymes and molecular) for this paper.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683058			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the 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 or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the feed for 4 weeks.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	15 birds were used in each treatment group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
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<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. Lipids 15(3):151-156.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683058			
Domain	Metric	Rating	Comments	
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest (food consumption), but the results were summarized for the treatment groups and not by individual animal.
	Metric 18:	Consistency of Outcome Assessment	High	
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	Low	
	Metric 23:	Explanation of Unexpected Outcomes	Low	A measure of variability is not reported in Figure 1.
Additional Comments:	This form is for the data provided for feed consumption behavior. This is a behavioral endpoint, but it also a confounding variable in avian dietary toxicity studies			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. <i>Lipids</i> 15(3):151-156.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683058			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the 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 or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the diet for 4 weeks.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was tested.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors report that 15 chickens were used per treatment group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. <i>Lipids</i> 15(3):151-156.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683058			
Domain	Metric	Rating	Comments	
Domain 6: Confounding / Variable Control				
Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall.	
Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior).	
Domain 7: Data Presentation and Analysis				
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.	
Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment and control group as % egg production, but no measure of variability was presented.	
Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report a measure of variability for egg production.	
Additional Comments:	This evaluation is for the egg production endpoint.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. <i>Lipids</i> 15(3):151-156.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	683058		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The chemical was identified by name.
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and the methods for preparation of the 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 or measurements were not reported.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type. Animals were exposed through the diet for 4 weeks.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was tested.
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via diet.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Authors reported that there were 15 animals used per treatment.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of 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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<b>Study Citation:</b>	Wood, D. L., Bitman, J. (1980). The effect of feeding di-(2-ethylhexyl) phthalate (DEHP) on the lipid metabolism of laying hens. <i>Lipids</i> 15(3):151-156.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus domesticus</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	683058		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior). Because they report that weight was not affected, the confounding effects of this behavioral change may be minor overall.
Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors reported that the treatments affected food consumption compared to the control (aversion behavior).
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	Medium	There do not appear to be any unexpected outcomes, and variability for body weight was reported. No measure of variability was reported for liver weight.
Additional Comments: This evaluation form is for the growth endpoints of body and liver weight.			

**Overall Quality Determination****High**

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

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

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249807			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.7%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1249807

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 outcomes unrelated to exposures among study groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form was for hatch defects.

**Overall Quality Determination**

**High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249807			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	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 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	High	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1249807		
Domain	Metric	Rating	Comments
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	High	There were no outcomes unrelated to exposures among study groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This mortality form was added for hatch.			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249807			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical purity was reported as 99.7%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one test concentration and a control were used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1249807		
Domain	Metric	Rating	Comments
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	High	There were no outcomes unrelated to exposures among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation form is for activity and imprinting.			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249807			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical substance 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 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	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one test concentration and a control were used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via egg injection.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to the maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Gallus gallus domesticus</i> ; Cobb broiler strain; Embryo		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	1249807		
Domain	Metric	Rating	Comments
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	High	There were no outcomes unrelated to exposures among test groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation form was for DNA damage.			
<b>Overall Quality Determination</b>		<b>High</b>	

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

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<b>Study Citation:</b>	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Ovis aries</i> ; Manchega breed; Adult			
<b>Health Outcome:</b>	Mechanistic-Endocrine toxicity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519005			
Domain	Metric	Rating	Comments	
Domain 5: Outcome Assessment				
Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.	
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—changes in estradiol and progesterone levels.	
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Enzyme immunoassay kits were used to determine hormone levels.	
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups.	
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
Metric 21:	Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.	
Metric 22:	Reporting of Data	High	Exposure and control data were presented in Figures 1 and 2 and were adequate for the outcomes of interest.	
Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability was reported in the figures.	
Additional Comments:	This portion of the evaluation was on the effect of DEHP on plasma estradiol and progesterone levels. The endocrine mechanistic outcome was chosen as the outcome of interest.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Ovis aries</i> ; Manchega breed; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519005			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was not reported, nor was it analytically verified.	
	Metric 3: Test Substance Purity	Low	The DEHP purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using six sheep as negative controls that received saline injections.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Figures 1 and 2.	
	Metric 6: Randomized Allocation	Medium	Sheep were randomly allocated into control or treatment groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The preparation of the DEHP injections was not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures were administered via intramuscular injection 3x a week for 2 months. Other details regarding the exposure administration were not reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the exposure concentration was measured.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The goal of the study was not to have a dose response, but to observe one treatment level and compare it to a control.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via injection.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the sheep were acclimated prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Ovis aries</i> ; Manchega breed; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519005			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—changes in the ovaries due to DEHP exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Ovarian structures were examined via ultrasound, and after that, they were fixed and examined via light microscopy.
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.
	Metric 22:	Reporting of Data	High	Exposure and control data were presented in Figures 1 and 2 and were adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes. Variability was reported in the figures.
<b>Additional Comments:</b>	This portion of the evaluation was on the effect of DEHP on ovarian structure in Manchega sheep. Reproduction was selected as the outcome of interest.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

<b>Study Citation:</b>	Herreros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Ovis aries</i> ; Manchega breed; Adult			
<b>Health Outcome:</b>	Hepatic/Liver			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519005			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the DEHP was not reported, nor was it analytically verified.	
	Metric 3: Test Substance Purity	Low	The DEHP purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using six sheep as negative controls that received saline injections.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Figure 3.	
	Metric 6: Randomized Allocation	Medium	Sheep were randomly allocated into control or treatment groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The preparation of the DEHP injections was not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures were administered via intramuscular injection 3x a week for 2 months. Other details regarding the exposure administration were not reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the exposure concentration was measured.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 2 months with intramuscular injections 3x a week. After the exposure period, the follicular phase was observed. This appeared to be an adequate duration to observe a response.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The goal of the study was not to have a dose response, but to observe one treatment level and compare it to a control.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via injection.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Sheep were reported to be from the experimental farm of the INIA in Madrid, Spain. It was reported adult, female sheep of the Manchega breed were used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the sheep were acclimated prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 6 sheep in the control and 8 sheep in the treatment group. This is a small sample size.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Herrerros, M. A., Encinas, T., Torres-Rovira, L., Garcia-Fernandez, R. A., Flores, J. M., Ros, J. M., Gonzalez-Bulnes, A. (2013). Exposure to the endocrine disruptor di(2-ethylhexyl)phthalate affects female reproductive features by altering pulsatile LH secretion. Environmental Toxicology and Pharmacology 36(3):1141-1149.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Injection			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Ovis aries</i> ; Manchega breed; Adult			
<b>Health Outcome:</b>	Hepatic/Liver			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2519005			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions and feeding of the sheep.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—changes in liver tissue.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Liver tissue was fixed and examined via light microscopy.	
Domain 6: Confounding / Variable 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 or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical methods were described in the "statistical analysis" section.	
	Metric 22: Reporting of Data	High	Exposure and control data were presented in Figure 3 and were adequate for the outcomes of interest.	
	Metric 23: Explanation of Unexpected Outcomes	High	The study authors did not report any unexpected outcomes.	
Additional Comments:	This portion of the evaluation was on the effect of DEHP on liver structure in Manchega sheep. The hepatic/liver was selected as the outcome of interest.			

**Overall Quality Determination****Medium**

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

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<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	746754			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups. There were 6 control animals & 7 DEHP-treated animals.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on the results. The outcome assessment methods were detailed and were appropriate for the outcomes of interest.
	Metric 17:	Outcome Assessment Methodology	High	
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported & were assessed consistently (after organisms were killed at conclusion of study).
Domain 6: Confounding / Variable 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 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 (e.g., infection) that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Student's T test was used.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This study did not adequately measure the animals' intake of DEHP, rendering it unacceptable.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult		
<b>Health Outcome:</b>	Hepatic/Liver		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	746754		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].
	Metric 2: Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British Petroleum Chemicals International Ltd., Epsom, Surrey."
	Metric 3: Test Substance Purity	High	DEHP was reported to have >99% purity, which was verified by GLC.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A concurrent negative control was used.
	Metric 5: Negative Control Response	High	Biological responses in the negative control were normal.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.
	Metric 8: Consistency of Exposure Administration	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.
	Metric 9: Measurement of Test Substance Concentration	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.
	Metric 10: Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	This was a single dose study (1% DEHP w/w diet).
	Metric 12: Testing at or Below Solubility Limit	N/A	This was a dietary exposure.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	"Male albino ferrets ( <i>Putorius putorius</i> ) were obtained from the Wellcome Veterinary Research Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually mature."
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
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<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult			
<b>Health Outcome:</b>	Hepatic/Liver			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	746754			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods were detailed and were appropriate for the outcomes of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, & were assessed consistently (after organisms were killed at conclusion of study).	
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21: Statistical Methods	High	Student's T test was used.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This study did not adequately measure the animals' intake of DEHP, rendering it unacceptable.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	746754		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The chemical was identified by the accepted name [Di-(2-ethylhexyl)-phthalate (DEHP)].
	Metric 2: Test Substance Source	High	"DEHP ("Bisoflex DOP") was obtained from British Petroleum Chemicals International Ltd., Epsom, Surrey."
	Metric 3: Test Substance Purity	High	The DEHP purity was >99%, and it was verified by GLC.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A concurrent negative control was used.
	Metric 5: Negative Control Response	High	Biological responses of the negative control were normal.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Uninformative	Dietary dosing was listed as 1% DEHP by weight, but the amount of food given per day was not listed. Accordingly, "The mean daily intake of DEHP was 1200 mg/kg/day, but owing to the seasonal fluctuation in the body weight of the ferret (see ref. 15 and Fig. 1) the daily DEHP intake ranged from 650 to 2000 mg/kg." Because the dose was not measured, this study is unusable for dose response, but provides useful evidence for hazard ID.
	Metric 8: Consistency of Exposure Administration	Uninformative	See Metric 7. The dose of DEHP was not measured, and it was stated to vary throughout the study.
	Metric 9: Measurement of Test Substance Concentration	Uninformative	See Metrics 7 & 8. The dose was not measured, and it varied throughout the study.
	Metric 10: Exposure Duration and Frequency	High	This was a daily dietary exposure for 14 months.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	This was a single dose study (1% DEHP w/w diet).
	Metric 12: Testing at or Below Solubility Limit	N/A	This was a dietary exposure.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	"Male albino ferrets ( <i>Putorius putorius</i> ) were obtained from the Wellcome Veterinary Research Station, Frant, Kent. The animals were 18 months of age at the commencement of the experiment (body weight range 1150–1850 g) and were judged to be sexually mature."
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
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<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	746754			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Frequency of weighing was not given & can only be estimated from Fig. 1.	
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were confusing, limited, or not reported.	
Domain 6: Confounding / 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 or other non-treatment-related factors across study groups. In particular, the loss of body weight from DEHP-treated food given ad libitum begs the question of palatability, and whether animals consumed a similar amount of control food.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Student's T test was used.	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	This study did not adequately measure the animals' intake of DEHP, rendering it unacceptable.			

**Overall Quality Determination****Uninformative**

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

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<b>Study Citation:</b>	Lake, B. G., Brantom, P. G., Gangolli, S. D., Butterworth, K. R., Grasso, P. (1976). Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. Toxicology 6(3):341-356.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Putorius putorius</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Cytotoxicity-Cardiovascular-Endocrine toxicity-Gastrointestinal-Kidney/renal-Liver toxicology-Neurotoxicology-Reproductive/Teratogenic-Respiratory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	746754			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 control animals & 7 DEHP-treated animals.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Conditions were not reported, but there were no indications that differences would have a significant impact on results.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methods were detailed and were appropriate for the outcomes of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, & were assessed consistently (after organisms were killed at conclusion of study).
Domain 6: Confounding / 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 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 Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	The cell morphology assessment didn't need statistical analysis.
	Metric 22:	Reporting of Data	Medium	Some of the cell morphology results were shown in figures. More was described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This study did not adequately measure the animals' intake of DEHP, rendering it unacceptable.			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Streptopelia risoria</i> ; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	681729			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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	
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 Characterization				
	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 Administration	Low	
	Metric 9:	Measurement of Test Substance Concentration	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 was used to assess eggshell thickness, weight, rate of water loss, surface area, and permeability.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study does 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 dove pairs and replicates used were not reported. But it was reported that a total of 34 eggs were used to determine eggshell thickness index, weight, rate of water loss, surface area and permeability (Table 1).

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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Avian; <i>Streptopelia risoria</i> ; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	681729		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	Pairs of ring doves were maintained as described in Peakall,1970. A feeding schedule was not provided.
Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodologies were briefly described. References were cited for eggshell index calculation (Ratcliffe, D.A., 1970) and permeability determination (HERO ID: 2180519; pdf not available).
Metric 18:	Consistency of Outcome 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 / 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 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 Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for the treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	No details were given for the dietary toxicity testing methods. The duration of exposure and exposure frequency, and the number of pairs of doves used in the experiment were not reported.		

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	Correct nomenclature was reported, but no CASRN or figure of the structure were provided.	
Metric 2:	Test Substance Source	Low	The source of the chemical substance was listed as Sigma Aldrich, but the substance was not analytically verified.	
Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Control groups were administered with water.	
Metric 5:	Negative Control Response	High	Control hormone responses were reported and adequate for every timepoint (Figure 2).	
Metric 6:	Randomized Allocation	Medium	The authors reported random allocation of test animals to treatment groups.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods were described, but the preparation of the treatment concentrations did not detail the preparation of the oral gavage.	
Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among treatment and control groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual exposure concentration was reported.	
Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was over four weeks when piglets were three weeks of age. This duration and exposure age was targeting the impacts of DEHP on the development of the HPG-axis (GnRH-stimulation). To examine the function of the HPG-axis, the boars were stimulated at the end of the recording period (nine months of age) with a GnRH analogue and the hormone concentrations in blood were measured.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).	
Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form was administered via a gavage to pure DEHP.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	The source (experimental station at Lovsta, Gotland, Sweden) and strain (cross bred, Swedish Yorkshire x Swedish Landrace) were reported.	
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<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain	Metric	Rating	Comments	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Animal care was well described in section 2.1.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Eight DEHP-exposed and eight control boars were used for the GnRH-stimulation test (section 2.4). However, due to malfunctioning catheters, data from the GnRHstimulation post-stimulation was based on seven animals from each group (section 3.2 and Fig. 2). There were no replicate groups.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many care considerations are mirrored in 850.2400.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology (GnRH-stimulation) addressed the intended outcome of interest (to examine DEHP impacts on the function of the HPG-axis) but representation (Fig. 2) of the assessment methodology should have been more clear. Furthermore, the authors reported catheter problems during the experiment, and thus, it is hard to interpret the one time-point display of significant differences in LH concentration between control and treatment groups at 30 minutes.	
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was reported and assessed consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No environmental conditions were indicated that could alter treatment and control animals.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost. There were also "sporadic cases of diarrhea" that were treated with IM-injections of trimethoprim-sulphonamide to the affected animals.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Authors indicated that parameters were analyzed using the Mixedprocedure of SAS ( <a href="http://support.sas.com/">http://support.sas.com/</a> ). Data collected during the hour before the GnRH-stimulation were analyzed separately from the data after the GnRH-stimulation. Furthermore, paired t-tests were used to investigate the effects of DEHP on GnRH-stimulation. But authors did not report if adjustments were conducted for multiple statistical tests that were run (ie Bonferroni test/correction).	
	Metric 22: Reporting of Data	High	Results were provided in Figure 2.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	Problems with blood collection and catheters reduced the sample size for both treatment and control groups to seven individuals.	

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

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Correct nomenclature was given, but no CASRN or figure of the structure were provided.
	Metric 2:	Test Substance Source	Low	The source of the chemical substance was listed as Sigma Aldrich, but the substance was not analytically verified.
	Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control groups were administered with water.
	Metric 5:	Negative Control Response	High	Responses of the reproductive behavior endpoints were adequate for the control group (Tables 1, 2, and 3).
	Metric 6:	Randomized Allocation	Medium	The authors report random allocation of test animals to treatment groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods were described, but the preparation of the treatment concentrations did not detail the preparation of the oral gavage.
	Metric 8:	Consistency of Exposure	High	Exposures were consistent among treatment and control groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual exposure concentration.
	Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was 4 weeks when piglets were 3 weeks of age. This duration of exposure age was to examine the impacts of DEHP on mating behavior of male boars from 6 to 9 months of age.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).
	Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form administered via a gavage to pure DEHP.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source (experimental station at Lovsta, Gotland, Sweden) and strain (cross bred, Swedish Yorkshire x Swedish Landrace) were reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Animal care was well described in section 2.1.
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<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	8 control and 8 DEHP individuals were used for the mating behavior/reproductive behavior experiments, which were conducted for 14 weeks (from 6-9 months of age). There were no replicate groups.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many care considerations are mirrored in 850.2400.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mounting counts, time to mount, attempts to ejaculate) was reported and appropriate for the intended outcome of interest (to examine DEHP impacts on reproductive behavior).
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was reported and assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No environmental conditions indicated that could alter treatment and control animals.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost; as a result, the surviving paired siblings were removed from the study, which was most appropriate. There were also "sporadic cases of diarrhea" that were treated with IM-injections of trimethoprim-sulphonamide to the affected animals; however, there were no details explaining if these sporadic cases were equally prevalent in the control or DEHP group or if there is evidence for the sporadic cases affecting the outcomes of interest.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors indicate "mixed procedure" in SAS after inspecting for normality and homogeneity of variance. Chi-square tests were conducted on reproductive behavior (Table 1: mating behavior counts; Table 2: mating behavior duration; Table 3: Percentage mounts & ejaculation).
	Metric 22:	Reporting of Data	High	All behavior data are presented within Tables 1-3.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were noted by the authors and measure of variability (SE) was provided.
Additional Comments:	This form was used to evaluate the reproductive behavior impacts of DEHP on mating behavior of male boars from 6 to 9 months of age.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	Correct nomenclature was reported, but no CASRN or figure of the structure were provided.	
Metric 2:	Test Substance Source	Low	Source was listed as Sigma Aldrich but was not analytically verified.	
Metric 3:	Test Substance Purity	High	Authors reported purity from Sigma as 99.5%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Control groups were administered with water.	
Metric 5:	Negative Control Response	High	The biological response (plasma concentrations of LH, oestradiol and testosterone at 30 minute intervals for 6 hours) before and during the 4 weeks of repeated (three times weekly) administration of 300 mg/kg DEHP was reported and adequate for the control group (Fig. 1).	
Metric 6:	Randomized Allocation	Medium	The authors report random allocation of test animals to treatment groups.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods were described, but the preparation of the treatment concentrations did not detail the preparation of the oral gavage.	
Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent among treatment and control groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Dietary treatment concentrations were reported as nominal. No analytical verification of actual concentration.	
Metric 10:	Exposure Duration and Frequency	High	The dietary exposure was over 4 weeks when piglets were 3 weeks of age. This duration and exposure age was adequate for the study type (the impacts of DEHP, endocrine disrupter, on the development of the HPG-axis).	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to have a dose-dependent effect and there was only one exposure concentration (300 mg/kg body weight of DEHP) which was based on a previously published study at a lower concentration from the same lab group (Hero ID 6566145).	
Metric 12:	Testing at or Below Solubility Limit	N/A	Compound in a dietary form administered via a gavage to pure DEHP.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	The source (experimental station at Lovsta, Gotland, Sweden) and strain (cross bred, Swedish Yorkshire × Swedish Landrace) were reported.	
Metric 14:	Acclimatization and Pretreatment Conditions	High	Animal care and pretreatment conditions were well described in section 2.1.	
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<b>Study Citation:</b>	Ljungvall, K., Spjuth, L., Hulten, F., Einarsson, S., Rodriguez-Martinez, H., Andersson, K., Magnusson, U. (2006). Early post-natal exposure to low dose oral di(2ethylhexyl) phthalate affects the peripheral LH-concentration in plasma, but does not affect mating behavior in the post-pubertal boar. Reproductive Toxicology 21(2):160-166.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Juvenile			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683666			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	Plasma concentrations of LH, oestradiol and testosterone (Fig. 1): authors reported using 20 individual animals per group (DEHP or placebo (water)). There were no replicate groups.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The authors adequately detailed care, handling, and exposure procedures in 2.1. Many care considerations are mirrored in 850.2400.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (radioimmunoassay to measure plasma hormone concentrations) was reported and adequate for the intended outcome of interest (impacts of DEHP on concentrations of reproductive hormones in plasma).
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was reported and assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No environmental conditions indicated that could alter treatment and control animals.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The end of section 2.1 indicated that a control and treatment individual were lost; as a result, the surviving paired siblings were removed from the study, which was most appropriate. There were also "sporadic cases of diarrhea" that were treated with IM-injections of trimethoprim-sulphonamide to the affected animals; however, there were no details explaining if these sporadic cases were equally prevalent in the control or DEHP group or if there is evidence for the sporadic cases affecting the outcomes of interest.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors indicate "mixed procedure" in SAS after inspecting for normality and homogeneity of variance. All data used in the Mixed procedure were log-transformed to improve the normal distribution and the homogeneity of variances. The least square option was used to compare different means at different time-points for the hormonal data.
	Metric 22:	Reporting of Data	High	Figure 1 presents exposure related findings adequately.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were noted by the authors and measure of variability (SE) was provided.
Additional Comments:	This form was used to evaluate the impacts of DEHP on sex hormone (testosterone, oestradiol 17-beta, and LH) concentrations in the plasma in piglets during the 4 week exposure of DEHP (Figure 1).			

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<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus domesticus</i> ; Swedish Yorkshire x Swedish landrace; Juvenile		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	683666		
Domain	Metric	Rating	Comments
<b>Overall Quality Determination</b>		<b>High</b>	

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

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

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes (DEHP-exposed animals had slightly lower rates of spermatozoa morphological abnormalities than controls) were thoroughly explained (small effect size that disappeared by the time boars were eight months old, possible artifact of subjective sperm quality assays).

Additional Comments: This study examined the semen and sperm quality effects of orally-administered pure DEHP (300 mg/kg bw) to domestic boars several months after exposure. A comprehensive suite of semen and sperm quality parameters was evaluated in a high-quality lab that serves as the reference laboratory for semen evaluation in Sweden. Assessed semen parameters included ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity. All findings have been evaluated in this form because all parameters were examined in detail and no negative effects of early oral exposure to DEHP on the post-pubertal sperm output and sperm quality were found. This form is for the mechanistic outcome of sperm plasma membrane integrity.

**Overall Quality Determination****High**



<b>Study Citation:</b>	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshire x Swedish Landrace; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	683808			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Chemical was identified by nomenclature but structure and CASRN were not provided.	
	Metric 2: Test Substance Source	High	Obtained from Sigma-Aldrich, Stockholm, Sweden.	
	Metric 3: Test Substance Purity	High	99.5% purity.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	One sibling from each of ten pairs was randomly assigned to control.	
	Metric 5: Negative Control Response	High	One mortality in negative controls, from endocarditis (resulting in 10% mortality rate). There was one mortality in DEHP treated group as well, from myositis. The corresponding sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the experiment was therefore 16 (eight in each group).	
	Metric 6: Randomized Allocation	Medium	Explicitly stated that one pig from each pair, with pairs taken from 10 separate litters, was randomly assigned to DEHP or control group.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Pure DEHP was administered orally via blunt syringe.	
	Metric 8: Consistency of Exposure Administration	High	Administration was the same for all groups, including control (i.e., placebo administration of same amount of water).	
	Metric 9: Measurement of Test Substance Concentration	Medium	Dietary exposure was to the pure compound via blunt syringe, but the DEHP dose of 300 mg/kg (i.e. 0.3 mL/kg) body weight was not measured/analytically verified.	
	Metric 10: Exposure Duration and Frequency	High	Exposure thrice weekly from 3 weeks of age to 7 weeks of age (1 month exposure) in pre-pubertal boars was appropriate to examine the effects of DEHP exposure on sperm quality later in life (up to 9 months of age).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The study goal was not to determine a dose-response relationship.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was oral administration of the pure compound.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Piglets were obtained from research station stock and reared at the Swedish University of Agricultural Sciences, Uppsala, Sweden.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all piglets were weaned at 3 weeks postpartum and from then until 5 months of age were housed in two boxes, one for each group (DEHP or control). Also, they reported that one month before collection of semen started, i.e. at five months of age, the animals were moved to individual pens with straw bedding at the Division of Comparative Reproduction, Obstetrics and Udder Health, SLU, Uppsala, Sweden.	
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<b>Study Citation:</b>	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshire x Swedish Landrace; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	683808

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	10 piglets were used in each group. Until 5 months of age, all piglets were housed in two boxes, one for each group(DEHP or control). At 5 months of age, the animals were moved to individual pens.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Details of husbandry conditions were minimal (temperature, feed amount and frequency, pen size were not reported), but there are no details in the study to indicate that conditions would have an impact on results.
	Metric 17: Outcome Assessment Methodology	High	The methodologies to evaluate ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity were appropriate to examine sperm quality impacts later in life following exposure during pre-puberty). The methodologies employed for sperm quality examination were appropriate and well-detailed.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / 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 one mortality in each group of control and treatment from endocarditis and myositis, respectively; for these two pigs, the corresponding paired sibling in the other treatment group was excluded from the experiment and the remaining number of animals in the study was 16 (8 per group). Also, there were sporadic cases of diarrhea that was treated with intramuscular trimethprim-sulfonamide if animal condition was affected, but no details on were reported on whether controls or treatment animals were more affected.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described (nested model run in SAS).
	Metric 22: Reporting of Data	High	Data for treatment & control presented.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes (DEHP-exposed animals had slightly lower rates of spermatozoa morphological abnormalities than controls) were thoroughly explained (small effect size that disappeared by the time boars were 8 months old, possible artifact of subjective sperm quality assays).

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<b>Study Citation:</b>	Spjuth, L., Ljungvall, K., Saravia, F., Lundeheim, N., Magnusson, U., Hulten, F., Rodriguez-Martinez, H. (2006). Does exposure to di(2-ethylhexyl) phthalate in pre-pubertal boars affect semen quality post-puberty?. International Journal of Andrology 29(5):534-542.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Vertebrate; Mammalian; <i>Sus scrofa</i> ; Swedish Yorkshire x Swedish Landrace; Juvenile
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	683808

Domain	Metric	Rating	Comments
Additional Comments:	This study examined the semen and sperm quality effects of orally-administered pure DEHP (300 mg/kg bw) to domestic boars several months after exposure. A comprehensive suite of semen and sperm quality parameters was evaluated in a high-quality lab that serves as the reference laboratory for semen evaluation in Sweden. Assessed semen parameters included ejaculate volume, sperm concentration, total sperm count, individual sperm motility, sperm morphology, and sperm membrane integrity. All findings have been evaluated in this form because all parameters were examined in detail and no negative effects of early oral exposure to DEHP on the post-pubertal sperm output and sperm quality were found.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . <i>Environmental Pollution</i> 251:871-878.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5593882			
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 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. A solvent control 0.1% DMSO was used.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."	
	Metric 8: Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S-basal medium containing E. coli OP50 at 20 C for 72 h to reach adult stage."	
	Metric 9: Measurement of Test Substance 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 (reproduction/development).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were minor limitations regarding the number of exposure groups (2).	
	Metric 12: Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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.	
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<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . Environmental Pollution 251:871-878.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5593882

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20: Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$ . Survival curves were analyzed using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments: This evaluation is for brood size.			

**Overall Quality Determination****Medium**

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

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

**Overall Quality Determination****Medium**

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

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

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . <i>Environmental Pollution</i> 251:871-878.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5593882			
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	Low	Purity or grade of test substance were not reported	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."	
	Metric 8: Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S-basal medium containing <i>E. coli</i> OP50 at 20 C for 72 h to reach adult stage."	
	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 (metabolic response)	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	The solvent concentration was appropriate (0.1% DMSO)	
	Metric 12: Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	all pretreatment conditions were the same for control and exposed organisms	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . Environmental Pollution 251:871-878.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5593882			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome 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
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$ . Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained
Additional Comments:	defecation cycle, pharyngeal pump			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5555457		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."
Metric 2:	Test Substance Source	Low	DEHP was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). It was not analytically verified prior to test initiation.
Metric 3:	Test Substance Purity	Low	Purity and grade were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Negative solvent control (1% DMSO) was used.
Metric 5:	Negative Control Response	High	Control responses were adequate for the test.
Metric 6:	Randomized Allocation	Low	The allocation procedure was not reported.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	The concentration was not measured during the study, nor was there any discussion on how loss of test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.
Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent for all groups.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
Metric 10:	Exposure Duration and Frequency	High	Exposure duration was appropriate for the test.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and spacing were adequate for the test. "Synchronized wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with <i>E. coli</i> OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."
Metric 12:	Testing at or Below Solubility Limit	High	DEHP was added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.
Domain 4: Test Organism			
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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5555457			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	The organisms used were hermaphroditic. Their life stage and age were well described. "Wild-type N2 <i>C. elegans</i> was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L cholesterol) that were seeded with a lawn of <i>Escherichia coli</i> OP50 as a food source in a 20 °C incubator. Synchronization of <i>C. elegans</i> was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchronized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S-basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to the test substance.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each test group contained at least five worms. Replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate for the test.	
	Metric 17: Outcome Assessment Methodology	High	The intended outcome (reproduction) was reported.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.	
	Metric 20: Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean ± standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05."	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	

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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5555457

Domain	Metric	Rating	Comments
Additional Comments:	For Metric 7. Experimental System/Test Media Preparation: Nominal concentrations only were reported from the onset of the test. Given the hydrophobic nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable. This form is for the mechanistic outcomes reported in Fig. 6 for the F0 generation to the F5 generation.		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5555457			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."	
Metric 2:	Test Substance Source	Low	DEHP purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). It was no analytically verified prior to test initiation.	
Metric 3:	Test Substance Purity	Low	Purity and grade were not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Negative solvent control (1% DMSO) used.	
Metric 5:	Negative Control Response	High	Control response were adequate for the test.	
Metric 6:	Randomized Allocation	Low	Allocation procedure not reported.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	Concentration not measured during study, nor was there any discussion on how loss test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.	
Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent for all groups.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
Metric 10:	Exposure Duration and Frequency	High	Exposure duration was appropriate for test.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing were adequate for the test. "Synchronized wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with E. coli OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."	
Metric 12:	Testing at or Below Solubility Limit	High	DEHP added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.	
Domain 4: Test Organism				
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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5555457			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	Organisms are hermaphrodite, life stage and age well described. "Wild-type N2 <i>C. elegans</i> was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L cholesterol) that were seeded with a lawn of <i>Escherichia coli</i> OP50 as a food source in a 20 °C incubator. Synchronization of <i>C. elegans</i> was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchronized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S-basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to test substance.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each test group contained at least 5 worms. Replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate for test.	
	Metric 17: Outcome Assessment Methodology	High	Intended outcome (reproduction) was reported.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.	
	Metric 20: Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean ± standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05."	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5555457

Domain	Metric	Rating	Comments
Additional Comments:	For Metric 7. Experimental System/Test Media Preparation: Nominal concentrations only were reported from the onset of the test. Given the hydrophobic nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5555457			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	There was no indication of the CAS number or other identifying information other than it was di(2-ethylhexyl) phthalate (DEHP). "DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration."	
	Metric 2: Test Substance Source	High	DEHP purchased from Sigma-AldrichChemicals Co. (St. Louis, MO, USA)	
	Metric 3: Test Substance Purity	Low	Purity and grade were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative solvent control (1% DMSO) used.	
	Metric 5: Negative Control Response	High	Control response were adequate for the test.	
	Metric 6: Randomized Allocation	Low	Allocation procedure not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Concentration not measured during study, nor was there any discussion on how loss test substance was minimized. Minimal information was provided. This criteria is borderline unacceptable.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were consistent for all groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration was appropriate for test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing were adequate for the test. "Synchronized wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with E. coli OP50 (109 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors."	
	Metric 12: Testing at or Below Solubility Limit	High	DEHP added to cell culture media (agar). The solvent, DMSO, was added to enhance solubility.	
Domain 4: Test Organism				
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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5555457			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	Organisms are hermaphrodite, life stage and age well described. "Wild-type N2 <i>C. elegans</i> was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH2PO4, 1.7% agar, 0.25% peptone, 1 mM CaCl2, 1 mM MgSO4, 5 mg/L cholesterol) that were seeded with a lawn of <i>Escherichia coli</i> OP50 as a food source in a 20 °C incubator. Synchronization of <i>C. elegans</i> was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchronized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S-basal (100 mM NaCl, 50 mM KH2PO4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988)."	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms, such that the only difference was exposure to test substance.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each test group contained at least 20 worms. Replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate for test.	
	Metric 17: Outcome Assessment Methodology	High	Intended outcome (behavior) was reported.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were consistently assessed across all groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.	
	Metric 20: Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure were reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were well described. "Statistical analysis was performed using SPSS Statistics 22.0 (IBM, Inc., New York, USA, 2013). The results were presented as the mean ± standard error of mean (SEM) values. One-way ANOVA with Tukey's test as the post hoc test was used to determine the statistical difference between means unless otherwise stated. Differences were considered statistically significant at p b 0.05."	
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest. Negative findings were reported.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.	
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<b>Study Citation:</b>	Li, S. W., How, C. M., Liao, V. H. (2018). Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in <i>Caenorhabditis elegans</i> . <i>Science of the Total Environment</i> 634:260-266.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild-type N2; Larvae
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5555457

Domain	Metric	Rating	Comments
Additional Comments:	For Metric 7. Experimental System/Test Media Preparation: Nominal concentrations only were reported from the onset of the test. Given the hydrophobic nature of DEHP in addition to a lack of analytical confirmation, this study is not completely reliable.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.	
	Metric 2: Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.	
	Metric 3: Test Substance Purity	High	Purity from source (sigma) listed at 99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A solvent control (0.01% DMSO) was used.	
	Metric 5: Negative Control Response	High	Figure 1 (page 4/8) lists the control responses for target genes. Control gene expression was considered 1, with treatment concentrations demonstrating relative expression normalized by the control response.	
	Metric 6: Randomized Allocation	Low	No random allocation was described.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.	
	Metric 8: Consistency of Exposure Administration	High	The two treatment concentrations appear to be administered similarly.	
	Metric 9: Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, concentrations are reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	A 48 hr duration for this gene expression bioassay appear to be appropriate for <i>C. elegans</i> .	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50 value for this species.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration and replicates were not reported in the methods but in figure 1 caption, it states "n=6" for each treatment concentration.	
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences that would indicate confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.
	Metric 22:	Reporting of Data	High	Relative expression is reported in figure 2 (page 4/8) for the target genes.
	Metric 23:	Explanation of Unexpected Outcomes	High	Standard Error of the Mean are reported for each treatment concentration and gene.
Additional Comments:	Expression of lipid metabolism and stress response genes were assessed by exposing worms at L1 stage to 1 and 10 mM DEHP for 48 h.”Genes associated with lipid metabolism, including <i>fasn-1</i> , <i>pod-2</i> , <i>fat-5</i> , <i>acs-6</i> and <i>sbp-1</i> , and vitellogenin were upregulated. Among the stress response genes, <i>ced-1</i> <i>wah-1</i> , <i>daf-21</i> and <i>gst-4</i> were upregulated, while <i>ctl-1</i> , <i>cdf-2</i> and the heat shock proteins ( <i>hsp-16.1</i> , <i>hsp-16.48</i> and <i>sip-1</i> ) were downregulated”.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.
	Metric 2:	Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) listed at 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.
	Metric 5:	Negative Control Response	High	Figure 3 (page 5/8) lists the control responses for body area effects.
	Metric 6:	Randomized Allocation	Low	No random allocation was described.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.
	Metric 8:	Consistency of Exposure Administration	High	The two treatment concentrations appear to be administered similarly.
	Metric 9:	Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, concentrations are reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	A 48 hr duration for the assessment of body area appears to be appropriate for <i>C. elegans</i> .
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50 value for this species.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).
	Metric 15:	Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration were not reported in the methods but are available in figure 3 as "n=30" for each treatment concentration. No replication was reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.
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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	4728405

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No reported differences that would indicate confounding variables.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.
	Metric 22: Reporting of Data	High	Data and results describing the body area assessment are shown in Figure 3C and in the text on page 379.
	Metric 23: Explanation of Unexpected Outcomes	Low	Scatter plots are used to represent the body area among treatment concentrations, so traditional error terms are not available.

Additional Comments: The body area measurements indicated that there was no significant difference in *C. elegans* development upon exposure to DEHP (Fig. 3C).

## Overall Quality Determination

## High



<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of the compound listed.
	Metric 2:	Test Substance Source	Low	The source was listed, but the performing lab did not analytically verify the test substance.
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	A concurrent negative control group was not included or reported.
	Metric 5:	Negative Control Response	Low	The results did not state the results of the control for the survival bioassay (first paragraph of section 3).
	Metric 6:	Randomized Allocation	Low	No random allocation was described.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.
	Metric 8:	Consistency of Exposure Administration	High	The four treatment concentrations appeared to be administered similarly.
	Metric 9:	Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	A 48-hr duration for this acute bioassay appeared to be appropriate for <i>C. elegans</i> .
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The concentrations were selected above and below a previously published LC50 value for this species.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Twenty stage L1 worms were used per replicate and there were five replicates per treatment concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.

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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	"the survival assay which did not show any lethality at 0.1, 1, 10 and 100 mM of DEHP and DEP over 48 h time period (data not shown)."
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences that would indicate confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Negative findings occurred across all groups. "the survival assay which did not show any lethality at 0.1, 1, 10 and 100 mM of DEHP and DEP over 48 h time period (data not shown)."
	Metric 22:	Reporting of Data	Uninformative	The authors presented no data on the mortality study.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not report any data for these mortality assays and did not expect the treatment concentrations to result in no mortality.
Additional Comments: A concurrent negative control group was not included or reported and data for mortality was not reported for any treatment group.				

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.	
	Metric 2: Test Substance Source	Low	Source listed, performing lab did not analytically verify the test substance.	
	Metric 3: Test Substance Purity	High	Purity from source (sigma) listed at 99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A solvent control (0.01% DMSO) was used.	
	Metric 5: Negative Control Response	High	Figure 3 (page 5/8) lists the control responses for fat staining are presented with color intensity and body area.	
	Metric 6: Randomized Allocation	Low	No random allocation was described.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.	
	Metric 8: Consistency of Exposure Administration	High	The two treatment concentrations appear to be administered similarly.	
	Metric 9: Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, concentrations are reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	A 48 hr duration for this fat staining bioassay appear to be appropriate for <i>C. elegans</i> .	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The two concentrations (1 and 10 uM) were selected below a previously published LC50 value for this species.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment. Rearing media before and during testing was the same (minus the compound).	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers per treatment concentration were not reported in the methods but are available in figure 3 as "n=30" for each treatment concentration. No replication was reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.	
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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Nutritional & Metabolic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences that would indicate confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett's post-hoc.
	Metric 22:	Reporting of Data	High	The presentation of 'relative Nile red intensity' for fat staining is presented in figure 3." Lipid quantification was done by measuring the fluorescence intensity of the individual worms and normalizing the values to their respective body area".
	Metric 23:	Explanation of Unexpected Outcomes	Low	Scatter plots are used to represent the fat staining among treatment concentrations, so traditional error terms are not available.
Additional Comments: Results of lipid staining assay (Fig 3) showed that DEHP significantly increased lipid content following 1 mM exposure.				
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	698288			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name
	Metric 2:	Test Substance Source	High	Source identified as Sigma-Aldrich.
	Metric 3:	Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	High	exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups were adequate for a dose response
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test animals was not reported
	Metric 14:	Acclimatization and Pretreatment 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 characterize 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

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<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	698288			
Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted..
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes,
Additional Comments:	Landfill soils also tested			

**Overall Quality Determination****Uninformative**

<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	698288			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Chemical was identified by name	
	Metric 2: Test Substance Source	High	Source identified as Sigma-Aldrich.	
	Metric 3: Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	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	Medium	The duration of exposure was reported and was adequate	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups were adequate for a dose response	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported	
	Metric 14: Acclimatization and Pretreatment 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 characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	

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<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	698288		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups
Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted..
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes,
Additional Comments: Landfill soils also tested			
<b>Overall Quality Determination</b>		<b>Uninformative</b>	



<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	698288			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	High	The source was identified as Sigma-Aldrich.	
	Metric 3: Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported.	
	Metric 14: Acclimatization and Pretreatment 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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	698288		
Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not conducted.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: Landfill soils were also tested.			
<b>Overall Quality Determination</b>		<b>Uninformative</b>	

<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	698288			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Chemical was identified by name	
	Metric 2: Test Substance Source	High	Source identified as Sigma-Aldrich.	
	Metric 3: Test Substance Purity	High	Pure analytical-grade DEHP was used in the experiment	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	High	exposures were administered consistently across study groups.	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
	Metric 10: Exposure Duration and Frequency	Medium	The duration of exposure was reported and was adequate	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups were adequate for a dose response	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test animals was not reported	
	Metric 14: Acclimatization and Pretreatment 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 characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
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<b>Study Citation:</b>	Roh, J., Jung, I., Lee, J., Choi, J. (2007). Toxic effects of di(2-ethylhexyl)phthalate on mortality, growth, reproduction and stress-related gene expression in the soil nematode <i>Caenorhabditis elegans</i> . <i>Toxicology</i> 237(1-3):126-133.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type Bristol Strain; Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	698288

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups
	Metric 20: Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were not shown for each treatment and control group
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes,

Additional Comments: Landfill soils also tested

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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 <i>C. elegans</i> . <i>PLoS Genetics</i> 15(2):e1007975.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5043459			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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				
	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 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	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/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 Organism				
	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 Assessment				
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<b>Study Citation:</b>	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 <i>C. elegans</i> . <i>PLoS Genetics</i> 15(2):e1007975.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Larvae
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Genotox (including DNA repair)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5043459

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	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 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 strains were tested, but it is unclear which strain results were reported. This form was for the DEHP mechanistic outcome reported in Fig. 1A.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae			
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	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.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure 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	Medium	The duration of exposure was reported and adequate for the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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, and repeated tests were used as replicates (n=3).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae			
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test 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 adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation form is for ethanol pretreatment and AFD thermosensory neurons (fig.7).				

**Overall Quality Determination****High**



<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 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	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	Medium	The duration of exposure was reported and adequate for the study type	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	Only one concentration tested	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via culture medium	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 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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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments: ethanol pretreatment, locomotor and thermotactic, fig.6			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 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	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	Medium	The duration of exposure was reported and adequate for the study type	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	Only one concentration tested	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via culture medium	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 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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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments: Ascorbic acid pretreatment, locomotor and thermotactic, fig.6			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured
	Metric 10:	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 Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 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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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described
	Metric 22: 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 acid pretreatment, reactive oxygen species			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8: Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
	Metric 10: Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	Only one concentration tested	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via culture medium	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 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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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Genotox (including DNA repair)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments: gene expression, fig.4			
<b>Overall Quality Determination</b>		<b>High</b>	



<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8: Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
	Metric 10: Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate for a dose response	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via culture medium	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 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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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments: locomotor and thermotaxis			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured
	Metric 10:	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 Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 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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<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; wild type; Larvae		
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described
	Metric 22: 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: Ethanol pretreatment, reactive oxygen species			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae			
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	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.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Only one concentration was tested.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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, and repeated tests were used as replicates (n=3).
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.
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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae		
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint of interest.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation form is for ascorbic acid pretreatment and AFD thermosensory neurons (fig.7).			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae			
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215375			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	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.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure 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	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 Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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, and repeated tests were used as replicates (n=3).
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.

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<b>Study Citation:</b>	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 <i>Caenorhabditis elegans</i> . PLoS ONE 8(12):e82657.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; DA 1267; Larvae		
<b>Health Outcome:</b>	Mechanistic-Neurotoxicology-Ocular and Sensory		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215375		
Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: 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: This evaluation form is for assessment of AFD thermosensory neurons.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829298			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	Source was reported but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was 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. Concentrations of test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported but without adequate details. Solvent concentration used was not reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	Only a 24 hr exposure but it was suitable to determine non-toxic levels.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were suitable but did not elicit a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility but solvents at an appropriate level aided in dissolution. Biological response of solvent control was adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported but experiments were repeated. Reporting regarding number of test organisms was confusing. "Thirty L4 nematode larvae were exposed to 24 h for BPA and DEHP at five concentrations of 100, 10, 1, 0.1 and 0.01 mg/L" It was stated that each experiment was repeated three times but data presented only for 30 larvae (Fig 1.).	

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<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	4829298

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions of test system were minimally reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	Medium	Incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confounding / Variable 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 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 adequately.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group and results were described in the text. Y-axis label of Fig 1 a) showing mortality data is incorrect.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: No effect of DEHP on mortality at the concentrations tested.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cytotoxicity-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829298			
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 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	The biological response of the negative control groups was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported but not in sufficient detail.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration was suitable to determine a dose response.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility, but solvents at an appropriate level aided in dissolution. The solvent concentration used was not reported, but biological response of the solvent control was adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported but experiments were repeated.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of the test system were minimally reported.	
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<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cytotoxicity-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829298			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies (number of apoptotic cells in oocytes, gene expression of apoptosis and oxidative stress related genes, HUS- 1 protein expression, H2O2 levels and DNA damage) reported the intended outcomes of interest.
	Metric 18:	Consistency of Outcome Assessment	Medium	There was incomplete reporting of minor details of outcome assessment protocol execution.
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 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 adequately.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group and results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This evaluation form is relevant for all the mechanistic endpoints -number of apoptotic cells in oocytes, gene expression of apoptosis and oxidative stress related genes, HUS- 1 protein expression, H2O2 levels and DNA damage in <i>C. elegans</i> exposed to 0.1, 1 and 10mg/L of DEHP.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829298			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	Source was reported but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Concentrations of test substance were not measured during the study	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported but not in sufficient detail.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	The duration was for 24 hours.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Concentrations exceeded solubility but solvents at an appropriate level aided in dissolution. The solvent concentration used was not reported but the biological response of solvent control was adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported but experiments were repeated. The total number of test organism for each reproductive assay were as follows- 15 nematodes were used for brood size and generation time. 20 nematodes were used to count oocytes, 30 nematodes were used for observing gonadal structure but number of nematodes used per treatment were not provided.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Yin, J., Liu, R., Jian, Z., Yang, D., Pu, Y., Yin, L., Wang, D. (2018). Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in <i>Caenorhabditis elegans</i> . <i>Ecotoxicology and Environmental Safety</i> 163:298-306.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; N2 Wild Type; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4829298			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions of test system were minimally reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (for brood size, generation time, number of oocytes per gonad arm and observing gonadal structure) reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	Medium	Incomplete reporting of minor details of outcome assessment protocol execution
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 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 adequately.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were shown for each treatment and control group (Fig 1 and Fig 2) and results were described in the text.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	This evaluation form covers a number of reproductive endpoints (brood size, generation time, gonadal structural changes and oocyte count) for <i>C. elegans</i> exposed to 10, 1, and 0.1 mg/L of DEHP.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . <i>Environmental Pollution</i> 251:871-878.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5593882			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group. A solvent control 0.1% DMSO was used.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. "DEHP was prepared and diluted in dimethyl sulfoxide (DMSO) to make 1000-fold stock solutions. The final concentration of DMSO in each treatment was 0.1% as solvent control."	
	Metric 8: Consistency of Exposure Administration	Low	Few details of exposure administration were reported. "L1-stage wild-type N2 worms were exposed to 0 (0.1% DMSO as solvent control) and 1.5 mg/L DEHP in liquid S-basal medium containing E. coli OP50 at 20C for 72 h to reach adult stage."	
	Metric 9: Measurement of Test Substance 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 (mortality).	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	The solvent concentration was appropriate (0.1% DMSO).	
	Metric 12: Testing at or Below Solubility Limit	High	A solvent was used to enhance the solubility of DEHP.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	How, C. M., Yen, P. L., Wei, C. C., Li, S. W., Liao, C., V.H. (2019). Early life exposure to di(2-ethylhexyl)phthalate causes age-related declines associated with insulin/IGF-1-like signaling pathway and SKN-1 in <i>Caenorhabditis elegans</i> . <i>Environmental Pollution</i> 251:871-878.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild Type N2; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5593882			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately. "Paired comparisons were conducted using Student's t-test. Multiple comparisons were done with one-way ANOVA followed with Tukey's post hoc test by using SPSS 22.0 (IBM, Inc., New York, USA, 2013). Two-way ANOVA was used to assess significance and interaction between DEHP exposure and age of the worms (days of adulthood) on age-related endpoints. Statistically significant difference was defined at $p < 0.05$ . Survival curves were analyze using Mantel-Cox log-rank test by using GraphPad Prism (Graph-Pad Software, CA, USA)."
	Metric 22:	Reporting of Data	Low	Continuous data were presented without measures of variability.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments:	Metric 22: Reporting of data. This criteria could be considered unacceptable. No LC/EC 50 was established or indicated by the study authors. This data is strictly informational in nature and could not be used quantitatively in a RE.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of the compound listed.
	Metric 2:	Test Substance Source	Low	The source was listed, but the performing lab did not analytically verify the test substance.
	Metric 3:	Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control (0.01% DMSO) was used.
	Metric 5:	Negative Control Response	High	Control responses for total progeny per day within the 5-day study are reported in Figure 4A.
	Metric 6:	Randomized Allocation	Low	No random allocation was described.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.
	Metric 8:	Consistency of Exposure Administration	Medium	The one treatment concentration appeared to be administered similarly.
	Metric 9:	Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal.
	Metric 10:	Exposure Duration and Frequency	High	The 5-day duration for progeny production seems to capture fecundity in this species.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	The study only examined one treatment concentration (1 uM). No justification was given for just using a single concentration.
	Metric 12:	Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment and section 2.5 details the handling of animals for fecundity. Rearing media before and during testing was the same (minus the compound).
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was one animal per plate and 24 animals for the treatment concentration and corresponding control.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences that would indicate confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors indicate that ANOVA was performed with Dunnett’s post-hoc.
	Metric 22:	Reporting of Data	High	The presentation of total progeny per day is presented in figure 4A.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Scatter plots are used for total progeny, so no variance terms are presented.
Additional Comments:	The study only examined one treatment concentration (1 uM) and exposure concentration was not measured. No justification was given for using a single concentration. DEHP reduced the fecundity at 1 mM concentration.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Other (please specify below) (Lifespan)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Authors have the CAS number, molecular weight, and structure of compound listed.	
	Metric 2: Test Substance Source	Low	Source was listed, but the performing lab did not analytically verify the test substance.	
	Metric 3: Test Substance Purity	High	Purity from source (sigma) was listed at 99.5%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A solvent control (0.01% DMSO) was used.	
	Metric 5: Negative Control Response	Medium	Control responses for lifespan were reported in the narrative as 14 days on page 6/8. No variance in this metric was reported.	
	Metric 6: Randomized Allocation	Low	No random allocation was described.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation was described adequately in section 2.1.	
	Metric 8: Consistency of Exposure Administration	Medium	The one treatment concentration appeared to be administered similarly.	
	Metric 9: Measurement of Test Substance Concentration	Low	No compounds were measured for analytical verification of treatment concentrations, and concentrations were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The 20 day duration for lifespan seems to be appropriate.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	The study only examined one treatment concentration (1 uM). No justification was given for just using a single concentration.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The growth media for <i>C. elegans</i> is a solid.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source was reported as from the Karolinski Institute. The animal care was well described.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Section 2.2 details animal care prior to treatment and section 2.5 details the handling of animals for lifespan. Rearing media before and during testing was the same (minus the compound).	
	Metric 15: Number of Organisms and Replicates per Group	Low	There was one animal per plate and 24 animals for the treatment concentration and corresponding control.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Pradhan, A., Olsson, P. E., Jass, J. (2018). Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of <i>Caenorhabditis elegans</i> . Chemosphere 190:375-382.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Caenorhabditis elegans</i> ; Wild type Bristol-N2; Larvae			
<b>Health Outcome:</b>	Other (please specify below) (Lifespan)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	4728405			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental variables during the test (temp, light cycle) were not reported, but should not impact results.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodology was appropriate for recording outcomes from the treatment concentrations.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be assessed consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences that would indicate confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was presented to indicate attrition or health outcomes that would interfere with the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Authors used a Mantel-Cox test to determine if lifespan was significantly different from control lifespan. (page 6/8).
	Metric 22:	Reporting of Data	Medium	The presentation of survival (lifespan) is presented in figure 4B.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No variance terms for lifespan were reported.
Additional Comments:	The study only examined one treatment concentration (1 uM). No justification was given for using a single concentration. Lifespan analysis indicated that DEHP reduced the average lifespan from 14 days in unexposed worms to 13 days in exposed worms.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5495570			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.	
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions could have a substantial impact on results.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	The duration of exposure was not clearly reported.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	There was only one exposure concentration with an appropriate solvent concentration.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if adequate.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest.	
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<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . <i>Basic &amp; Clinical Pharmacology &amp; Toxicology Online Pharmacology Online</i> 119(3):309-316.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo
<b>Health Outcome:</b>	Behavioral
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5495570

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions or other factors.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was presented to suggest differences in animal attrition or health outcomes that would impact results.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	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 evaluation is for food preference, CAFE, activity, and sleeping index.

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5495570			
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	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure 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	Low	Reporting omissions could have a substantial impact on results	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured	
	Metric 10: Exposure Duration and Frequency	Low	The duration of exposure was not clearly reported	
	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 diet	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if adequate	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5495570

Domain	Metric	Rating	Comments
	Metric 19: Confounding Variables in Test Design and Procedures	High	Nothing reported to indicate that environmental conditions or other factors influenced outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23: Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained

Additional Comments: Gene expression and circulating lipid/carbohydrate

## Overall Quality Determination

## Medium



<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5495570			
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	Low	Purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Low	Reporting omissions could have a substantial impact on results.	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	The duration of exposure was not clearly reported.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate..	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficient to evaluate if adequate.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
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<b>Study Citation:</b>	Cao, H., Wiemerslage, L., Marttila, P. S., Williams, M. J., Schiöth, H. B. (2016). Bis-(2-ethylhexyl) phthalate increases insulin expression and lipid levels in <i>Drosophila melanogaster</i> . Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online 119(3):309-316.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; CSORC wild-type; Embryo
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5495570

Domain	Metric	Rating	Comments
	Metric 19: Confounding Variables in Test Design and Procedures	High	No differences in environmental conditions or other non treatment conditions/factors were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	Authors did not report if there were no differences among groups
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23: Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained

Additional Comments: starvation resistance—presented in terms of survival hours in Figure 1, thus the mortality endpoint.

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . Chemosphere 221:493-499.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5495717		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	Identified by CASRN 117-81-7
	Metric 2: Test Substance Source	High	Sourced from Alfa Aesar
	Metric 3: Test Substance Purity	High	Purity given as "98+%"
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative control used.
	Metric 5: Negative Control Response	High	No unacceptable responses in control.
	Metric 6: Randomized Allocation	Medium	Authors reported randomized allocation.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Media preparation was detailed & adequate.
	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, and were only given as percentages (presumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administered media. If it is assumed that the percentages given are wet weights of total culture medium, the concentrations can be back-calculated from the given volumes and masses of constituents, but this is not certain.
	Metric 10: Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose-response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure via diet.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	Wild-type <i>D. melanogaster</i> of uncertain provenance.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	No report of acclimatization.
	Metric 15: Number of Organisms and Replicates per Group	Low	30-35 flies per replicate, number of replicates not reported.

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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . Chemosphere 221:493-499.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5495717		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Reporting insufficient to determine test conditions (only nominal temperature & photoperiod given).
	Metric 17: Outcome Assessment Methodology	Medium	Flies weighed in batches of 10 under anesthesia.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	One-way ANOVA tables provided.
	Metric 22: Reporting of Data	High	Data reported for all outcomes.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexplained outcomes reported.
Additional Comments: Because dose was not reported & cannot be back-calculated with confidence from the given information, this study is low-quality.			

**Overall Quality Determination****Low**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	Re-mating assay: Based on the description and results by the authors the biological response of the control group (re-mating rate of normal females with DEHP-untreated males, Fig 8B) was adequate.
Metric 6:	Randomized Allocation	Low	The authors did not report random allocation in the re-mating assay.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirm DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirm DEHP was dispersed after mixing, culture medium was examined by microscopy.
Metric 10:	Exposure Duration and Frequency	High	In Section 2.8, the authors reported that virgin flies were separated by sex and treated with 0, 0.1, 0.2, and 0.4% DEHP respectively, and that at day 10, the mating assays were performed. A 10-day exposure appeared adequate to study the response.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Re-mating assay: DEHP exposure groups were Ctrl (0%), 0.1, 0.2, and 0.4% DEHP as described in Section 2.8. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).	
	Metric 12: Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) was used in all assays.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.	
	Metric 15: Number of Organisms and Replicates per Group	Low	In Section 2.8, the authors reported using 24 replicates of naïve virgin female flies to mate with each DEHP-treated male one by one. However, it is not clear how many males they included in the study. Also, did they mean 24 female flies per treatment group? That is, it is unclear how many female and male flies were per group and how many treatment replicates there were.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (re-mating assay) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on reproductive system of males).	
	Metric 18: Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treatment 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 that would influence the outcome.	
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric		Rating	Comments
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 8B).
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figure 8B and Supplementary Table 11.
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.
<b>Additional Comments:</b>	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies ( <i>Drosophila melanogaster</i> ), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in <i>Drosophila</i> starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on <i>Drosophila</i> lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.			
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Larvae		
<b>Health Outcome:</b>	Neurological		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	Electrophysiology of <i>Drosophila</i> third-instar larvae neuromuscular junction: the biological response is expected to be adequate given the evidence in methodology provided by the authors.
Metric 6:	Randomized Allocation	Low	The study did not specifically state 'random allocation' or how organisms were allocated for the electrophysiological assessment of the neuromuscular junction in <i>Drosophila</i> larvae.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirm DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirm DEHP was dispersed after mixing, culture medium was examined by microscopy.
Metric 10:	Exposure Duration and Frequency	High	Exposure of 5 days was adequate to assess the electrophysiology of the neuromuscular junction as described by the authors.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Larvae			
<b>Health Outcome:</b>	Neurological			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Electrophysiological assessment of the neuromuscular junction: DEHP exposure groups were Ctrl (0%), 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).	
	Metric 12: Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Authors reported 11 (Control), 9 (0.2% DEHP), or 6 (0.4% DEHP) flies per treatment group in Figure 4, but they did not use treatment replicates per group. The number of flies in the high dose group was almost half of that in the control group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (electrophysiology of the neuromuscular junction) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on neural transmission).	
	Metric 18: Consistency of Outcome Assessment	High	Method details of the electrophysiology of the neuromuscular junction to evaluate neural transmission were reported and assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.	

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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Larvae
<b>Health Outcome:</b>	Neurological
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5494836

Domain	Metric	Rating	Comments
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for the post-hoc test (Figure 4).
	Metric 22: Reporting of Data	High	Data was reported for all treatment group the text and Figure 4 and Supplementary Tables 7 and 8.
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.

**Additional Comments:** Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (*Drosophila melanogaster*), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in *Drosophila* starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on *Drosophila* lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

**Overall Quality Determination****High**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
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<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	Gene expression in reproductive system of male <i>Drosophila</i> : Based on the description and results by the authors the biological response of the control group (mRNA expression of genes encoding proteins of the reproductive system) was adequate.
Metric 6:	Randomized Allocation	Medium	The authors indicated in Section 3.5 that <i>Drosophila</i> , male flies after eclosion were randomly divided into groups, and continuously supplied with culture medium containing a series of concentrations of DEHP respectively for 7 days.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 10:	Exposure Duration and Frequency	High	The authors reported in Section 3.5 that <i>Drosophila</i> , male flies after eclosion were randomly divided into groups, and continuously supplied with culture medium containing a series of concentrations of DEHP respectively for 7 days. A 7 day exposure is adequate to capture changes in gene expression.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Gene expression in reproductive system of male <i>Drosophila</i> : DEHP exposure groups were Ctrl (0%), 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Gene expression in reproductive system of male <i>Drosophila</i> : The number of test organisms and replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.
	Metric 17:	Outcome Assessment Methodology	High	The methodology used to measure mRNA expression (qPCR) was sufficiently described.
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treatment 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 across groups that would influence the outcome.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5494836

Domain	Metric	Rating	Comments
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 8A).
	Metric 22: Reporting of Data	High	Data was reported for all treatment group as per Figure 8A and Supplementary Table 10.
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.

**Additional Comments:** Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (*Drosophila melanogaster*), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in *Drosophila* starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on *Drosophila* lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

**Overall Quality Determination****High**

<b>Study Citation:</b>	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombination. <i>Mutagenesis</i> 8(1):57-81.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; y female x w male, Leiden Strain; Embryo			
<b>Health Outcome:</b>	Mechanistic-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	200657			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DEHP was identified by CASRN in Table VI.	
	Metric 2: Test Substance Source	Low	The DEHP was only reported to be commercially available; the source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity/grade of the DEHP was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The study authors reported using a concurrent negative control as seen in Table VI. The solvents used were 3% ethanol and 1% Tween.	
	Metric 5: Negative Control Response	High	The negative control response for DEHP was reported in Table VI and was adequate for the outcome of interest. All control results from unexposed larvae in all the exposures were reported in Table 1. The C-1 Eth./T control and pooled control results from all C-1 tests were reported here.	
	Metric 6: Randomized Allocation	Low	It was not reported how the <i>Drosophila</i> were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test concentrations and on the test system. It was reported that the test for DEHP was a chronic test in which 12-15 adult flies were placed in bottles and permitted to lay eggs for three days on food supplemented with the test substance dissolved in solvent before mixing into the standard food. Newly hatched females were transferred to fresh medium and scored 1-5 days later. Eyes of adult females were inspected for mosaic light spots under a dissecting scope.	
	Metric 8: Consistency of Exposure Administration	Low	Limited details were provided on the exposure system and the preparation of the test substance, so reporting omissions prevented a determination on consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point in the study.	
	Metric 10: Exposure Duration and Frequency	Low	The exact exposure duration was not reported. The chronic test reported that the adult flies were allowed to lay eggs onto treated media for three days. Newly hatched females were then transferred to fresh medium and their eyes were scored for mosaics 2-5 days later.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only two exposure groups, 10mM and 20mM, which is lower than is typical. Neither concentration reported a response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The exposure was via food, though the exposure could also have been via contact with the test substance in the food.	

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<b>Study Citation:</b>	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombination. <i>Mutagenesis</i> 8(1):57-81.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; y female x w male, Leiden Strain; Embryo			
<b>Health Outcome:</b>	Mechanistic-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	200657			
Domain	Metric	Rating	Comments	
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the <i>Drosophila</i> was not reported. It was reported strains y and w were from two different laboratory stocks, but it was unclear if those were cultured in house.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of eyes for the control and both the exposure groups was reported in Table VI. However, the number of replicates per exposure group was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions of the <i>Drosophila</i> . It is unclear what the temperature was and how large the exposure vessels were. They were fed, but the amount of food was not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—the number of adult female <i>Drosophila</i> that had mosaic eyes due to genotoxicity in comparison to the controls. Mosaic spots were classified according to size class, and analysis of clone size and distribution was reported.
	Metric 18:	Consistency of Outcome Assessment	Medium	It was reported that female <i>Drosophila</i> were assessed for mosaics 1-5 days after they were transferred to fresh media after they had hatched. This creates some inconsistency in when they were assessed, which may be due to when the eggs were oviposited. It was unlikely to have any substantial effect on the results.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Little information was provided on environmental conditions, nor was it reported if the organisms were acclimated.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A distinction was made regarding the size of the spots, and the total spots was reported. The frequency of clones per 10 <sup>4</sup> cells was calculated according to the formula f=2nm/NC, which is described in the text. The LED and HDT were calculated for active and inactive chemicals. A Chi-square test was reported to be used as statistical evaluation and test responses were categorized into strong responses, weakly positive responses, and negative responses.
	Metric 22:	Reporting of Data	High	Data for the DEHP exposure and the DEHP control were reported in Table VI, which was the inactive chemical table. The results were adequate for the outcome of interest. All control results for each test condition were provided in Table I.
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<b>Study Citation:</b>	Vogel, E. W., Nivard, M. J. (1993). Performance of 181 chemicals in a drosophila assay predominantly monitoring interchromosomal mitotic recombination. <i>Mutagenesis</i> 8(1):57-81.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; y female x w male, Leiden Strain; Embryo
<b>Health Outcome:</b>	Mechanistic-Genotox (including DNA repair)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	200657

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not provide any measures of variability even though average clone size and clones per 10 <sup>4</sup> cells were reported.

Additional Comments: This evaluation was on the effect of DEHP on genetic recombination in *Drosophila*. In the DEHP test, y males were crossed with w females and allowed to oviposit in bottles containing food supplemented with the test substance. Freshly hatched females were then transferred to fresh media and their eyes were assessed for mosaic spots 1-5 days later. Mechanistic genotoxicity was selected as the outcome of interest.

## Overall Quality Determination

## Medium



<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . Chemosphere 221:493-499.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5495717		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	Identified by CASRN 117-81-7
	Metric 2: Test Substance Source	High	Sourced from Alfa Aesar
	Metric 3: Test Substance Purity	High	Purity given as "98+%"
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative control used.
	Metric 5: Negative Control Response	High	No unacceptable responses in control.
	Metric 6: Randomized Allocation	Medium	Authors reported randomized allocation.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Media preparation was detailed & adequate.
	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, and were only given as percentages (presumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administered media. If it is assumed that the percentages given are wet weights of total culture medium, the concentrations can be back-calculated from the given volumes and masses of constituents, but this is not certain.
	Metric 10: Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose-response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure via diet.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	Wild-type <i>D. melanogaster</i> of uncertain provenance.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	No report of acclimatization.
	Metric 15: Number of Organisms and Replicates per Group	Low	30-35 flies per replicate, number of replicates not reported.

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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . Chemosphere 221:493-499.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5495717

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Reporting insufficient to determine test conditions (only nominal temperature & photoperiod given).
	Metric 17: Outcome Assessment Methodology	Medium	Dead flies were counted. <5% of flies escaped during the experiment & were excluded from the calculations of mortality.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	One-way ANOVA tables provided.
	Metric 22: Reporting of Data	High	Data reported for all outcomes.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexplained outcomes reported.
Additional Comments: Because dose was not reported & cannot be back-calculated with confidence from the given information, this study is low-quality.			

**Overall Quality Determination****Low**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . <i>Chemosphere</i> 221:493-499.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5495717			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by CASRN (117-81-7).	
	Metric 2: Test Substance Source	High	The test substance was sourced from Alfa Aesar.	
	Metric 3: Test Substance Purity	High	Authors reported the purity as "98+%"	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used.	
	Metric 5: Negative Control Response	High	There were no unacceptable responses in the control.	
	Metric 6: Randomized Allocation	Medium	Authors reported randomized allocation.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Media preparation was detailed & adequate.	
	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, and were only given as percentages (presumably of feed by wet weight?). Information was not sufficient to determine even the nominal concentration of the test substance in the administered media. If it is assumed that the percentages given are wet weights of the total culture medium. The concentrations can be back-calculated from the given volumes and masses of constituents, but this is not certain.	
	Metric 10: Exposure Duration and Frequency	High	Within the experiment, three different dosing frequencies/durations were tested: during the larval stage of the parents, for 24h, and for 20 days.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	Only three exposure concentrations were chosen, but results seem to indicate a dose-response relationship & are sufficient to estimate a LOAEC/NOAEC [or would be, if the concentrations were given].	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The study used wild-type <i>D. melanogaster</i> of uncertain provenance.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	There was no report of acclimatization.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 30-35 flies per replicate, but the number of replicates was not reported.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Cheng, J., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2019). Transgenerational impact of DEHP on body weight of <i>Drosophila</i> . Chemosphere 221:493-499.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Adult
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5495717

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Reporting was insufficient to determine test conditions (only nominal temperature & photoperiod given).
	Metric 17: Outcome Assessment Methodology	Medium	Emergence (reproductive success) was evaluated after five days.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	One-way ANOVA tables were provided.
	Metric 22: Reporting of Data	High	Data were reported for all outcomes.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported.

Additional Comments: Because dose was not reported & cannot be back-calculated with confidence from the given information, this study is low-quality.

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	Courtship assay measured by copulation duration and latency: Based on the description and results by the authors the biological response of the control group (duration and latency of copulation) was adequate.
Metric 6:	Randomized Allocation	Medium	The authors indicated in Section 3.4 that <i>Drosophila</i> male and female virgins were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP for 20 days.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirm DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirm DEHP was dispersed after mixing, culture medium was examined by microscopy.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 10:	Exposure Duration and Frequency	Medium	In the text (Section 3.4), the authors reported that <i>Drosophila</i> male and female virgins were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP for 20 days. However, Figure 7 indicates 10 days. Either 10 or 20 days exposure duration seemed adequate, but it is not clear what the actual duration was.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Courtship assay measured by copulation duration and latency: DEHP exposure groups were Ctrl (0%), 0.1%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).
	Metric 12:	Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daylight-shift.
	Metric 15:	Number of Organisms and Replicates per Group	Low	In Section 2.6, the authors reported at least 60 pairs of flies per group. It is not clear what the exact number of pairs per treatment was or how the number of pairs differed across groups.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (copulation latency and duration) was adequately described and was sensitive for the intended outcome of interest (DEHP effects on courtship behaviors).
	Metric 18:	Consistency of Outcome Assessment	High	There was no evidence indicating that outcomes were assessed differently across treatment groups.
Domain 6: Confounding / Variable Control				

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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Differences between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for post-hoc test (Figure 7).
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figure 7 and Supplementary Table 9.
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean.
Additional Comments:	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies ( <i>Drosophila melanogaster</i> ), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in <i>Drosophila</i> starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on <i>Drosophila</i> lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Ocular and Sensory		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	Function of the retina measured by the electroretinogram: the biological response was expected to be adequate given the evidence in methodology provided by the authors.
Metric 6:	Randomized Allocation	Low	The study did not specifically state 'random allocation' or how organisms were allocated for the assessment.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Finally, due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	Function of the retina measured by the electroretinogram: There is no evidence showing that exposure administration of DEHP (0%, 0.1%, and 0.4%) for 20 days was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.
Metric 10:	Exposure Duration and Frequency	High	The duration of the exposure of 20 days was adequate to evaluate whether DEHP exposure affects the function of the retina measured by the electroretinogram.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Ocular and Sensory			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Function of the retina measured by the electroretinogram: DEHP exposure groups: Ctrl (0%), 0.1%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016).	
	Metric 12: Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Authors reported 8 or 9 flies per treatment group and at least 6 replicate electroretinogram cycles for each fly, but they did not use treatment replicates per group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology (electroretinogram) addressed the intended outcome of interest (evaluation of the function of the retina).	
	Metric 18: Consistency of Outcome Assessment	High	Details of the electroretinogram methodology to evaluate function of the retina were reported and assessed consistently across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.	
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult
<b>Health Outcome:</b>	Ocular and Sensory
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	5494836

Domain	Metric	Rating	Comments
	Metric 21: Statistical Methods	High	Function of the retina measured by the electroretinogram: The difference between groups was calculated by one-way ANOVA and Dunnett's multiple comparison for the post-hoc test.
	Metric 22: Reporting of Data	High	Data was reported for all treatment group the text and Figure 3 and Supplementary Tables 5 and 6.
	Metric 23: Explanation of Unexpected Outcomes	High	The authors reported standard error of the mean (Figure 3).

**Additional Comments:** Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies (*Drosophila melanogaster*), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in *Drosophila* starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on *Drosophila* lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.

**Overall Quality Determination****High**

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified the test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	The maximum survival time of the last control group fly was reported to be 77 days for males and 82 days for females. The survival rate in the control group was adequate as a typical, healthy and well-maintained outbred <i>Drosophila</i> population will have a median lifespan of approximately 70 days and maximum of approximately 90 days at 25 °C.
Metric 6:	Randomized Allocation	Medium	The authors reported in the results section 3.1 that <i>Drosophila</i> within three days after eclosion were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirm DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured, and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, and culture medium was examined by microscopy.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 10: Exposure Duration and Frequency	High	The goal was to assess survival/mortality throughout the lifespan of <i>Drosophila</i> starting within three days after eclosion, while being continuously supplied with culture medium containing a series of concentrations of DEHP.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Mortality assessment: DEHP exposure groups: Ctrl (0%), 0.05%, 0.1%, 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016). Observation: The text in Section 2.3 indicates that doses were 0, 0.05, 1, 2, 4%, but it is most likely a typo because in Figures 1 and 2 and everywhere else in the article, the authors reported 0, 0.05, 0.1, 0.2, and 0.4% DEHP.	
	Metric 12: Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H day/night-shift.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 30-35 flies per replicate and seven replicates per treatment group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.	
	Metric 17: Outcome Assessment Methodology	Medium	The authors reported assessing mortality when the vials with culture medium for fly maintenance were replaced twice a week and that during the replacement some flies (<5%) escaped; flies that escaped were excluded from the calculation. It would have been most appropriate to determine and report how many flies per group were lost. While <5% might not be a high number, it is not clear if the authors made a general estimation that they were <5% or if they actually counted the number of flies that escaped.	
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	The outcome (mortality) was assessed consistently across study groups, but it is not clear if the <5% of flies that escaped applies to all treatment groups equally.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Mortality assessment: Authors used log-rank test and Gehan-Breslow-Wilcoxon test to determine the significance of the difference between survival curves.
	Metric 22:	Reporting of Data	High	Data was reported for all treatment groups in the text, Figures 1A and 1B, and Supplementary Tables 1 and 2.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The authors did not report the SE or CI.
Additional Comments:	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies ( <i>Drosophila melanogaster</i> ), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in <i>Drosophila</i> starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on <i>Drosophila</i> lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.			
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5494836		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	Authors identified test substance as bis(2-ethylhexyl) phthalate (DEHP) (over 98 %, Alfa Aesar, CAS-No: 117-81-7).
Metric 2:	Test Substance Source	Low	Authors reported the source of bis(2-ethylhexyl) phthalate (DEHP, over 98%, CAS-No: 117-81-7) as Alfa Aesar. But there was no analytical verification.
Metric 3:	Test Substance Purity	High	Authors reported the purity of bis(2-ethylhexyl) phthalate (DEHP, CAS-No: 117-81-7) as 98 plus %.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The authors reported using a control group with 0% DEHP.
Metric 5:	Negative Control Response	High	The motor function of the control group measured by the climbing assay showed an expected result and that the control group climbed for efficiently that the two highest groups.
Metric 6:	Randomized Allocation	Medium	The authors reported in the results section 3.1 that <i>Drosophila</i> within 3 d after eclosion were randomly divided into groups and continuously supplied with culture medium containing a series of concentrations of DEHP.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The control culture medium contained 10.52 g of agarose, 55.26 g of glucose, 27.57 g of sucrose, 47.85 g of cornmeal and 63.16 g of baker yeast in 1100 mL distilled water, with propionic acid (4.4 g dissolved in EtOH to final volume 10.63 mL). To prepare DEHP culture medium, DEHP (0.05, 0.1, 0.2, or 0.4% v/v) was added to the mixture containing ethanol. To confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy. However, while authors used ethanol (which brings DEHP into solution given that DEHP is insoluble in water), they did not specifically explain and describe the methods used to ensure that DEHP was present in the medium at the desired concentrations. Due to the non-volatile nature of DEHP, once present in the medium, there is no reason to believe that DEHP evaporated.
Metric 8:	Consistency of Exposure Administration	High	There is no evidence showing that exposure administration was not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured and the authors did not describe how they determined that the amount of ethanol used was sufficient to bring the desired amount of DEHP into the medium. At a minimum, to confirmed DEHP was dispersed after mixing, culture medium was examined by microscopy.
Metric 10:	Exposure Duration and Frequency	High	The goal was to evaluate motor function measured by climbing assay throughout the lifespan of <i>Drosophila</i> following continuous exposure to DEHP.
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . Environmental Pollution 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
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<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Motor function measured by climbing assay: DEHP exposure groups: Ctrl (0%), 0.05%, 0.1%, 0.2%, and 0.4%. Rationale offered by authors for the number and spacing of exposure groups: In rodents, DEHP has been tested form a very low dose of 0.015 mg/kg/day (Andrade et al., 2006) to a very high dose of 750 mg/kg/day (Gray Jr et al., 2000). Other toxicological studies with DEHP have examined exposure levels ranging from 10 to 100 mg/kg/day (Mu et al., 2015; Rajesh and Balasubramanian, 2015). In this study, the authors tested DEHP treatment on fruit flies from 0.05% to 0.4% (v/v), which is approximately 1.2-10mM or 35-300 mg/kg/day, with the approximation of 100 nL food consumption per day, and body weigh about 1 mg (Cao et al., 2016). Observation: The text in Section 2.3 indicates that doses were 0, 0.05, 1, 2, 4%, but it is most likely a typo because in Figure 2 and everywhere else in the article, the authors reported 0, 0.05, 0.1, 0.2, and 0.4% DEHP.	
	Metric 12: Testing at or Below Solubility Limit	Medium	DEHP is insoluble in water, and thus in <i>Drosophila</i> studies ethanol has been commonly used to bring DEHP into solution. While the control group (which had ethanol in the culture media) had an expected biological response in survival, it is unclear if the amount of ethanol used was sufficient to bring the desired DEHP concentrations into solution. Moreover, since DEHP was not measured, it is not clear how closely nominal concentrations were to actual concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Wild-type <i>Drosophila melanogaster</i> Canton Special (CS) wasused in all assays.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Authors reported that all flies (control or those assigned for DEHP groups) were kept in plastic vials at 25 C with 12H daynight-shift.	
	Metric 15: Number of Organisms and Replicates per Group	Low	30-35 flies per replicate and 7 replicates per treatment group at the start of the exposure. However, as flies died, it is not clear how many organisms were used per group in the actual climbing assay to evaluate motor function.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The housing conditions were typical of <i>Drosophila</i> cultures.	
	Metric 17: Outcome Assessment Methodology	Medium	To evaluate motor function/climbing activity, authors transferred the flies to a new vial and located them on the bottom of the vial on the culture medium, they agitated the vial and flies spontaneously started to climb upward along the vial. The authors reported that the whole process was video recorded and the number of flies that climbed over 5 cm within 15 s was counted. However, Figure 2 shows the climbing rate and the days (age) of the flies and the median and maximum climbing time. The data does not show the number of flies through time across treatment groups.	
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<b>Study Citation:</b>	Chen, M. Y., Liu, H. P., Liu, C. H., Cheng, J., Chang, M. S., Chiang, S. Y., Liao, W. P., Lin, W. Y. (2018). DEHP toxicity on vision, neuromuscular junction, and courtship behaviors of <i>Drosophila</i> . <i>Environmental Pollution</i> 243(Pt B):1558-1567.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Drosophila melanogaster</i> ; Canton Special; Adult			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494836			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	Low	The outcome (motor function/climbing activity) was assessed consistently across study groups, but it is not clear if the <5% of flies that escaped applies to all treatment groups equally. Moreover, the actual number of flies included in the motor function/climbing activity is unclear.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions across groups that would influence the outcome.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups, so the study received a Medium rating for this metric.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Motor function/climbing activity assessment: Authors used log-rank test and Gehan-Breslow-Wilcoxon test to determine the significance of the difference in motor function.	
	Metric 22: Reporting of Data	High	Data was reported for all treatment group the text and Figures 2A and 2B and Supplementary Tables 3 and 4.	
	Metric 23: Explanation of Unexpected Outcomes	Low	The authors did not report SE or CI of climbing assay but they reported the 95% CI of calculated hazard ratio (Supplementary Tables 3 and 4).	
Additional Comments:	Summary notes: The study evaluated the effects of DEHP exposure (1) on survival/mortality throughout the adult lifespan of fruit flies ( <i>Drosophila melanogaster</i> ), (2) on neurological impacts such as climbing motor skills and electrophysiology, (3) on ocular sensory measured by electroretinograms, and (4) on reproduction measured by a courtship assay, re-mating assay, and mRNA/gene expression (qPCR). Exposure duration and dose varied by endpoint/health outcome: Mortality and climbing motor skills were assessed in <i>Drosophila</i> starting exposure to DEHP (Ctrl (0%), 0.05, 0.1, 0.2, and 0.4%) within 3 d after eclosion and continuously supplied throughout the lifespan of the fruit flies (65-70 days); ocular sensory impacts were measured by electroretinogram following a 20 day exposure to DEHP (0, 0.1 and 0.4%); electrophysiological effects were measured following exposure to DEHP (0, 0.2, and 0.4%) from embryo to third instar stage (5 days); the courtship assay was performed after exposure to DEHP (0, 0.1, 0.4%) for 20 days or 10 days (it is unclear how many days exactly because in the text the authors reported 20 day exposure, but Figure 7 says 10 day exposure); the re-mating assay was performed after exposure to DEHP (0, 0.1, 0.2, 0.4%) for 10 days; gene expression (mRNA using qPCR) of genes important to reproduction was determined in males following exposure to DEHP (0, 0.2, 0.4%) for 7 days. Notes on <i>Drosophila</i> lifestages: Day 0: Female lays eggs; Day 1: Eggs hatch; Day 2: First instar (one day in length); Day 3: Second instar (one day in length); Day 5: Third and final instar (two days in length); Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying; Day 11-12: Eclosion (adults emerge from the pupa case). Females become sexually mature 8-10 hours after eclosion.			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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 <i>Eisenia fetida</i> . Journal of Environmental Quality 14(3):383-388.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	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)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eisenia fetida</i> ; Savigny; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3625226			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was listed by name and CAS number.
	Metric 2:	Test Substance Source	High	"The chemicals were purchased from the Aldrich Chemical Co., Milwaukee, WI, Eastman Kodak Co., Rochester, NY, and Fisher Scientific Co., Fairlawn, NJ."
	Metric 3:	Test Substance Purity	High	A minimum purity of 98% was stated.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls were utilized.
	Metric 5:	Negative Control Response	Low	A negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was unclear whether DEHP was dissolved in a solvent prior to addition to filter paper.
	Metric 8:	Consistency of Exposure Administration	Medium	It is unclear if a solvent was utilized and if the same amount of solvent was used across the different treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	This study utilized a 48-hr contact exposure duration to establish an LC50 value.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	Exposure concentrations were not stated. At least five concentrations were utilized for the definitive test (exact number of exposure groups not specified). It is unclear whether one of these concentrations was a control (blank).
	Metric 12:	Testing at or Below Solubility Limit	Low	The test substance was applied to filter paper using water or other solvent. It is unclear which was used and if concentrations exceeded solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not stated.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	An acclimation process/procedure was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 10 replicate worms per test concentration (1 worm per vial).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	It is unclear whether worms had adequate air flow during the exposure period.

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<b>Study Citation:</b>	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 <i>Eisenia fetida</i> . Journal of Environmental Quality 14(3):383-388.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	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)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Eisenia fetida</i> ; Savigny; Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	3625226

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	Mortality was assessed using a "gentle mechanical touch".
	Metric 18: Consistency of Outcome Assessment	High	Mortality was assessed in study groups after the 48-hour exposure duration.
Domain 6: 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	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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	"The LC50 value for each chemical tested was calculated using the method of Litchfield and Wilcoxon (1949)."
	Metric 22: Reporting of Data	Low	LC50 values with CI were reported in Table 1, but data for each individual treatment group was not shown.
	Metric 23: Explanation of Unexpected Outcomes	High	Confidence intervals were reported.

Additional Comments: This form is for the contact test. DEHP and DBP were not selected for the artificial soil test.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.	
	Metric 2: Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5: Negative Control Response	Low	The biological response of the negative control groups was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Experiments were conducted in small multidish chamber vessels with spiked soil. The exposure duration for this experiment was one week, and the experimental results for the degradation test (Figure 1) show that degradation after one week was greater than 20%. This may have an impact on results, though not as great as the impact for the longer experiments reported.	
	Metric 8: Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at beginning of experiment).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations were... DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight."	
	Metric 12: Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.	
Domain 4: Test Organism				
	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.	
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<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 juveniles per concentration, and two replications.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing, environmental conditions, food, water, nutrients and/or biomass loading was not sufficiently reported to evaluate if adequate and whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	Juveniles were assessed for survival after one week of exposure.	
	Metric 18: Consistency of Outcome Assessment	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 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 Presentation and Analysis				
	Metric 21: Statistical Methods	High	Details of the statistical methods used were reported in the methods section, and they were appropriate for the study.	
	Metric 22: Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualitatively. "Juvenile mortality was similar when exposed in multidishes with a hard soil surface and in microcosms with loose soil (data not shown)."	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	None			
Overall Quality Determination		Medium		

<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Test substance 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 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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Experiments were conducted in microcosm vessels with spiked soil. Because of the length of the exposure (22 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at beginning of experiment).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations used were DEHP at 0, 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg dry weight."	
	Metric 12: Testing at or Below Solubility Limit	N/A	Spiked soil exposure.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Male & female adult collembolans were used.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	No reported acclimatization, but no evidence to suggest results impacted.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	10 male & 10 female per microcosm, 4 replicates per concentration.	
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<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789786

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism housing & conditions acceptable. "Experiments were conducted at constant temperature (20oC), with a 12:12 h light: dark regime. Animals were fed dried baker's yeast (15 mg dry weight) at day 0 and day 14".
	Metric 17: Outcome Assessment Methodology	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 / 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 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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	Details of statistical methods reported in methods & are appropriate for the study.
	Metric 22: Reporting of Data	High	No effects were found at any concentration. EC10/LC10/EC50/LC50 are reported for all endpoints.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Concentration of DEHP in soil was not measured during or at the end of the experiment. Environmental conditions ( moisture content, pH, etc.) were not reported. There was no effect of DEHP on adult survival at the concentrations tested.		

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.	
	Metric 2: Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5: Negative Control Response	High	Biological responses in the control were normal.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Experiments were conducted in microcosm vessels with spiked soil. Because of the length of the exposure (22 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil. Concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at beginning of experiment).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations used were DEHP at 0, 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg dry weight."	
	Metric 12: Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Male and 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.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were ten males and ten females per microcosm, and four replicates per concentration.	
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Adult
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789786

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Organism housing and test conditions were acceptable. Experiments were conducted at a constant temperature (20C), with a 12:12 h light:dark regime. Animals were fed dried baker's yeast (15 mg dry weight) at day 0 and day 14.
	Metric 17: Outcome Assessment Methodology	Medium	Reproduction was assessed by counting surviving juveniles at the end of the study using digital image processing. However, juvenile mortality during the test period was not assessed, and was only counted at the end of the experiment.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups (at the end of the experiment).
Domain 6: Confounding / 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 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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	Details of statistical methods were reported in the methods and were appropriate for the study.
	Metric 22: Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualitatively or quantitatively. EC10/LC10/EC50/LC50 were reported for all endpoints.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Test substance 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 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 Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Experiments were conducted in small multidish chamber vessels with spiked soil. Because of the length of the exposure (60 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at beginning of experiment).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations were... DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight"	
	Metric 12: Testing at or Below Solubility Limit	N/A	Spiked soil exposure.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	A single 0 to 1-d old juvenile springtail was added to each chamber.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	No reported acclimatization, but no evidence to suggest results impacted.	
	Metric 15: Number of Organisms and Replicates per Group	Low	20 organisms per exposure concentration, no replicates.	
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<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789786

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism housing & conditions acceptable.
	Metric 17: Outcome Assessment Methodology	Medium	"During the first three weeks, covering an entire <i>F. fimetaria</i> life cycle, exuviae of growing juveniles were recorded every second day and removed if present." "Growth of the animals was determined manually at the screen by measuring the length from the posterior end of the abdomen to the anterior end of the head."
	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	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 Presentation and Analysis			
	Metric 21: Statistical Methods	High	Details of statistical methods reported in methods & are appropriate for the study.
	Metric 22: Reporting of Data	High	No effects were found at any concentration. EC10/LC10/EC50/LC50 are reported for all endpoints.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	789786			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified only by nomenclature. No other information (CASRN, structure etc.) was provided.	
	Metric 2: Test Substance Source	Low	"The chemicals used (DBP, DEHP, and acetone) were obtained from Merck Schuchardt, Darmstadt, Germany." The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used (acetone-water).	
	Metric 5: Negative Control Response	Low	The biological response of the negative control groups was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Experiments were conducted in small multidish chamber vessels with spiked soil. Because of the length of the exposure (60 days) loss of test substance is to be expected due to degradation, but no effort was made to replenish the phthalate in soil. Concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	DEHP concentrations were not measured throughout the study, and may be expected to degrade in soil. This may have a substantial impact on results.	
	Metric 10: Exposure Duration and Frequency	High	Exposure duration and frequency were acceptable (preparation of spiked soil at beginning of experiment).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	"Test concentrations were... DEHP at 0, 100, 250, 500, and 1,000 mg/kg dry weight."	
	Metric 12: Testing at or Below Solubility Limit	N/A	This was a spiked soil exposure.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	A single 0 to 1-d old juvenile springtail was added to each chamber.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	There was no reported acclimatization, but also no evidence to suggest results were impacted.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 20 organisms per exposure concentration, and no replicates.	
Domain 5: Outcome Assessment				

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<b>Study Citation:</b>	Jensen, J., Langevelde, van, J., Pritzl, G., Krogh, P. H. (2001). Effects of di(2-ethylhexyl) phthalate and dibutyl phthalate on the collembolan <i>Folsomia fimetaria</i> . Environmental Toxicology and Chemistry 20(5):1085-1091.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Folsomia fimetaria</i> ; Juvenile
<b>Health Outcome:</b>	Mortality
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	789786

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Organism housing and test conditions were acceptable. "Experiments were run at constant temperature (20oC), with a 12:12 h light:dark regime."
	Metric 17: Outcome Assessment Methodology	Medium	Juveniles were assessed twice a week for six weeks.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups (twice a week for six weeks).
Domain 6: Confounding / 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 (pH, moisture content, etc.) or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Details of the statistical methods used were reported in the methods, and were appropriate for the study.
	Metric 22: Reporting of Data	High	No effects were found at any concentration. Negative findings were reported qualitatively or quantitatively. EC10/LC10/EC50/LC50 are reported for all endpoints.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2345940			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS number.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical reported as pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.
Domain 3: Exposure 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	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via topical application.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Minor uncertainties about the characteristics of test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The use of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health although there were few details.
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<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2345940

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was performed and described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	There was no clear response.
Additional Comments: oxidative damage (Lipid peroxidation, TBARS)			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2345940			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS number.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical was reported as pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one test concentration was used (food choice test).
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via food choice, so solubility limit is not applicable.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There were minor uncertainties about the characteristics of the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	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 replicates were not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health, although there were few details provided.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

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<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dietary		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult		
<b>Health Outcome:</b>	Behavioral		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2345940		
Domain	Metric	Rating	Comments
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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was performed and described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: None			

**Overall Quality Determination****High**



<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2345940			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	Chemical was identified by name and CAS number.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Chemical reported as pure.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study group.	
Domain 3: Exposure 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	Exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via topical application.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Minor uncertainties about the characteristics of test organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The use of replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions of test system were conducive to maintenance of organism health although there were few details.	
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome of interest with few details.	

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<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Epigenetics
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2345940

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was not performed, results were assessed as levels of expression.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	There was no clear response.

Additional Comments: gene expression

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2347468			
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.	
	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 Characterization	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures were administered consistently across study groups.	
	Metric 9: 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/Spacing of Exposure Levels	N/A	Only one dose was used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	The test chemical was solubilized in methanol prior to topical application.	
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	Medium	There are minor reservations about the source of the test organisms.	
	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	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment	Metric 16: Adequacy of Test Conditions	Low	Minor uncertainties were identified regarding environmental conditions of the test system due to the lack of details provided.	
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 execution.
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 were no differences among groups, but there were few details to confirm that.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: 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 Quality Determination****Medium**

<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2345940			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS number.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical was reported as pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent and negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure 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	Exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one concentration was used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via topical application.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	There were minor uncertainties about the characteristics of test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The use of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were conducive to maintenance of organism health, although there were few details provided.

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<b>Study Citation:</b>	Cuvillier-Hot, V., Salin, K., Devers, S., Tasiemski, A., Schaffner, P., Boulay, R., Billiard, S., Lenoir, A. (2014). Impact of ecological doses of the most widespread phthalate on a terrestrial species, the ant <i>Lasius niger</i> . Environmental Research 131:104-110.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; N/A (e.g., injection); Dermal (topical application)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Lasius niger</i> ; Adult
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2345940

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	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 in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was performed and described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This evaluation is for egg laying rate.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Low	Unclear how the diet was spiked with the test material.	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food)and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	

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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Behavioral			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	The food consumption behavior results were shown in Figure 6.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were described in detail in section 2.8. Food consumption was analyzed with the Dunnet's Test.	
	Metric 22: Reporting of Data	Medium	Food consumption data was shown in Figure 6. Each data point represents a mean value without measures of variability.	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.	
Additional Comments:	Authors examined the effects of DEHP on food consumption behavior of <i>Spodoptera littoralis</i> . The exposure duration was not described explicitly but spanned the duration of development, and adult stage is about 5-10 days.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors noted that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Medium	Unclear how the diet was spiked with the test material.	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2	
	Metric 10: Exposure Duration and Frequency	Low	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from 4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP. However, since the concentration measured in food for the 1 ng/g condition was similar to the control one, they performed the measurement of DEHP contents only for the insects treated with 100 ng, 10 mg and 5 mg/g contaminated diet, and their controls.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . <i>Chemosphere</i> 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The authors reported in the figure caption (Figures 1 and2) that for analysis of DEHP in food, they used n = 6 to 11 for each condition; for analysis of DEHP in organisms, they used n = 3 - 4 biological replicates for each condition corresponding to a pool of 3 - 10 individuals).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	The authors measured concentrations of DEHP in various larval stages and confirmed concentrations in the control (background) and diet and in organisms using GC-MS.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were described in section 2.8 and the figure captions for Figures 1 and 2 indicate that statistical analyses were done. However, the authors did not indicate the type of analyses performed for the analytical measurement of DEHP.	
	Metric 22: Reporting of Data	High	DEHP concentrations in various larval stages and in the control (background) and diet are reported in Figures 1 and 2. They also conducted metabolomic profiling, where results are reported in the text and in Table 1.	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.	
Additional Comments:	The authors measured concentrations of DEHP in various larval stages and confirmed concentrations in the control (background) and diet. This form was used to evaluate the analytical measurements of DEHP in food and organisms (ADME).			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.
	Metric 2:	Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control (0.5% ethanol) was used.
	Metric 5:	Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.
	Metric 8:	Consistency of Exposure Administration	Medium	Unclear how the diet was spiked with the test material.
	Metric 9:	Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2.
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food)and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via diet.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.

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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors reported number of organism for the various endpoints in the figure captions. For the hemolymphatic analysis, authors reported n =7 to 12. For gene expression, the authors reported n = 6 for each condition in the figure caption. It is unclear if there were replicates.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	The mechanistic endpoints examined in this study were reported and sensitive to DEHP exposure. They included: hemolymphatic ecdysteroid titers and expression levels of ecdysteroid response genes (reported in Figures 7 and 8).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were described in section 2.8. Hemolymphatic concentrations of ecdysteroids and DEHP were compared using a Wilcoxon sum of rank test. qPCR results were analyzed with an ANOVA and Student t-test. Metabolomic results were analyzed using ANOVA and Student t-test.	
	Metric 22: Reporting of Data	High	For results for the hemolymphatic ecdysteroid titers and expression levels of ecdysteroid response genes are reported in figures 7 and 8.	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained	
Additional Comments:	The authors examined several mechanistic endpoints including whether DEHP treatments affect hemolymphatic ecdysteroid titers and expression levels of ecdysteroid response genes.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verify it analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Medium	It is unclear how the diet was spiked with the test material.	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS). Methods were described in section 2.2.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from 4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	

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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . <i>Chemosphere</i> 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	Mortality was reported from the 4th instar to the formation of the chrysalis.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were described in section 2.8. Mortality rates during larval and pupal stages were analyzed with a logistic regression test.	
	Metric 22: Reporting of Data	High	Cumulative mortality was reported for all treatments and control with a measure of variability (Figure 3).	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained.	
<b>Additional Comments:</b>	This form is specific to the results for cumulative mortality.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Low	Unclear how the diet was spiked with the test material	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from 4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	High	The developmental/growth outcomes reported include: the days to pupation (post embryonic development time), and weight of the larvae (Figures 4 and 6).	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analyses were described in detail in section 2.8. For development and growth outcomes: Sex-ratio was analyzed by comparison of proportions with a bilateral test (Monte Carlo method). Larval body mass changes and food consumption were analyzed with the Dunnet's Test. Durations of larval and pupal developmental stages were analyzed by one-way ANOVA.	
	Metric 22: Reporting of Data	Low	For the weight results, authors reported data in a figure as the mean weight without measures of variability.	
	Metric 23: Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained	
Additional Comments:	Authors examined the effects of DEHP on post embryonic development of <i>Spodoptera littoralis</i> . The exposure duration was not described explicitly but spanned the duration of development, and adult stage is about 5-10 days. Development/growth outcomes examined in this study include time to pupation (post embryonic development) and weight.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g. Sex ratio control data is included in Figure 5 and appears appropriate.	
	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 limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Low	It is unclear how the diet was spiked with the test material.	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from 4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food) and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . <i>Chemosphere</i> 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors report under the figure that n=35 larvae were used for each treatment, but it is unclear if each individual was treated as a replicate, or if the 35 animals were spread across technical replicates (chambers, jars, etc).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.
	Metric 17:	Outcome Assessment Methodology	High	Assessment of sex ratio was reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were described in detail in section 2.8. For development and growth outcomes: Sex-ratio was analyzed by comparison of proportions with a bilateral test (Monte Carlo method). Larval body mass changes and food consumption were analyzed with the Dunnet’s Test. Durations of larval and pupal developmental stages were analyzed by one-way ANOVA.
	Metric 22:	Reporting of Data	Medium	Results were reported in Figure 5 and described in the text. No raw data was shown.
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained and measures of variability were shown in Figure 5.
Additional Comments: This evaluation is for the assessment of sex ratio.				
Overall Quality Determination			Medium	

<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Endocrine toxicity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Di(2-ethylhexyl) phthalate (DEHP) was clearly identified but only by name. CASRN, structure, or other chemical descriptors were not reported.	
	Metric 2: Test Substance Source	Low	The chemical substance was obtained from PESTANAL® 36735, Sigma, France, but the authors did not verified analytically.	
	Metric 3: Test Substance Purity	Low	Purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Solvent control (0.5% ethanol) was used.	
	Metric 5: Negative Control Response	Medium	Control response was reported. The authors note that DEHP is present in the environment and food at low background concentrations - the diet of controls contained an average of 392 ± 125 ng of DEHP per g.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided limited details about how the doses in the diet were obtained, but a description was provided as to how doses were measured/validated.	
	Metric 8: Consistency of Exposure Administration	Medium	Unclear how the diet was spiked with the test material.	
	Metric 9: Measurement of Test Substance Concentration	High	Doses in the diet were confirmed analytically (GC-MS) - methods were described in 2.2.	
	Metric 10: Exposure Duration and Frequency	Low	The exposure duration was from the 3rd instar to pupation. The authors reported taking measurements in larvae, pupae (from4 to 6 days-old) and adults (2 days-old), but it is unclear how many days the study duration was.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors tested six environmentally relevant concentrations (100 pg; 1 ng; 10 ng; 100 ng; 1 mg and 10 mg per gram of food)and three high concentrations (500 mg, 5 mg and 40 mg per gram of food) of DEHP.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via diet.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized.	
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<b>Study Citation:</b>	Aviles, A., Boulogne, I., Durand, N., Maria, A., Cordeiro, A., Bozzolan, F., Goutte, A., Alliot, F., Dacher, M., Renault, D., Maibeche, M., Siaussat, D. (2019). Effects of DEHP on post-embryonic development, nuclear receptor expression, metabolite and ecdysteroid concentrations of the moth <i>Spodoptera littoralis</i> . Chemosphere 215:725-738.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Food/Diet; Dietary			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Spodoptera littoralis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Endocrine toxicity			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5494137			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	The authors reported number of organism for the various endpoints in the figure captions. For the hemolymphatic analysis, authors reported n =7 to 12. For gene expression, the authors reported n = 6 for each condition in the figure caption. It is unclear if there were replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Reporting of housing and environmental conditions and biomass loading was not sufficiently reported to evaluate whether differences occurred between control and exposed populations.
	Metric 17:	Outcome Assessment Methodology	High	The mechanistic endpoints examined in this study were reported and sensitive to DEHP exposure.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among groups outside of the exposure
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were described in section 2.8. Hemolymphatic concentrations of ecdysteroids and DEHP were compared using a Wilcoxon sum of rank test. qPCR results were analyzed with an ANOVA and Student t-test. Metabolomic results were analyzed using ANOVA and Student t-test.
	Metric 22:	Reporting of Data	High	For results for the hemolymphatic ecdysteroid titers are reported in Figure 7.
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were satisfactorily explained and measures of variability were shown.
Additional Comments:	This evaluation is for the assessment of ecdysteroid (hormones) levels.			
Overall Quality Determination			Medium	

<b>Study Citation:</b>	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the <i>Allium cepa</i> test. Mutation Research 743(1-2):20-24.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249401			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was identified by name and CASRN.	
	Metric 2: Test Substance Source	Low	The source was reported but the test substance was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and 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 was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and concentrations of test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance 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	The number of exposure groups and spacing of exposure levels were adequate to elicit a response in the mechanistic effects studied.	
	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 Organism				
	Metric 13: Test Organism Characteristics	High	The source of onion bulbs and the weight range (15-30g) were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were the same for control and exposed bulbs.	
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<b>Study Citation:</b>	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the <i>Allium cepa</i> test. <i>Mutation Research</i> 743(1-2):20-24.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Genotox (including DNA repair)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249401			
Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	5 bulbs were used for each concentration and control group. The use of replicates was not explicitly reported but in the data analysis section, it was stated that each data point represents mean ± standard deviation of three independent experiments. Assumption was made that the details in the experimental procedures section applies to the mitotic index and chromosome aberrations experiments.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Bulbs were grown in the dark at a constant temperature of 25± 0.5oC in an incubator. Bulbs were kept in glass receptacles filled with filtered tap water, which was aerated continuously. It was unclear whether these conditions (incubator at 25 oC) were maintained during exposure.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies for determining mitotic index and scoring of micronuclei and chromosome aberrations were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Mechanistic endpoints were measured after 48 hours of exposure and appears to be consistently done across treatment groups.
Domain 6: Confounding / 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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were clearly reported.
	Metric 22:	Reporting of Data	High	Cytogenetic alterations were given as mean ± standard deviation for each treatment group and control in Table 3. Statistical significance was also presented.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Mitotic activity in root meristems was first evaluated and based on the results, the concentrations that produced a mitotic index above 50% of the control was used to conduct subsequent experiments to determine cytogenetic alterations in meristematic cells.			
Overall Quality Determination			High	

<b>Study Citation:</b>	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the <i>Allium cepa</i> test. Mutation Research 743(1-2):20-24.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249401			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported but the test substance was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and grade of the test substance were not reported. Test purity was described as the "highest grade commercially available".
Domain 2: Test 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 was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations, and concentrations of the test substance were not measured during the study.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance 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	Uninformative	The number of exposure groups and spacing of exposure levels were not conducive to the purpose of the study. EC50 values could not be determined.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate. No effects on biological responses were observed in the solvent control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source of onion bulbs and the weight range (15-30g) were reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were the same for control and exposed bulbs.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Five bulbs were used for each concentration and control group. The use of replicates was not explicitly reported, but in the data analysis section it was stated that each data point represents mean ± standard deviation of three independent experiments.
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Herrero, O., Martín, Pérez, J. M., Freire, Fernández, P., López, Carvajal, L., Peropadre, A., Hazen, M. J. (2012). Toxicological evaluation of three contaminants of emerging concern by use of the <i>Allium cepa</i> test. Mutation Research 743(1-2):20-24.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1249401			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	Medium	Bulbs were grown in the dark at a constant temperature of 25± 0.5C in an incubator. Bulbs were kept in glass receptacles filled with filtered tap water, which was aerated continuously. It was unclear whether these conditions (incubator at 25C) were maintained during exposure.	
	Metric 17: Outcome Assessment Methodology	Low	The root length measurement method was not clearly reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 18: Consistency of Outcome Assessment	High	Root length was measured after 72 hours of exposure and appears to be consistent across treatment groups.	
Domain 6: Confounding / 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 (e.g., infection) that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were clearly reported.	
	Metric 22: Reporting of Data	High	Mean root length data ± standard deviation (presented as percentage of control values) was given for each treatment group and control.	
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
<b>Additional Comments:</b>	A dose-dependent effect of DEHP on growth (via measurement of root length) could not be established and EC50 values could not be determined.			
<b>Overall Quality Determination</b>		<b>Uninformative</b>		



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Chemical purity reported as 99.6%	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.	
Domain 4: Test 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 Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Allium cepa</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / 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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Allium cepa* with DEHP exposure.

## Overall Quality Determination

## High

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure 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 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 Conditions	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 characterize 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
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments: MDA"MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species."			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2915866		
Domain	Metric	Rating	Comments
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	Nothing was reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."		

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Avena sativa</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Avena sativa* with DEHP exposure.

**Overall Quality Determination** **High**



<b>Study Citation:</b>	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant <i>Benincasa hispida</i> and its use for lowering DEHP content of intercropped vegetables. <i>Journal of Agricultural and Food Chemistry</i> 61(22):5220-5225.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Air; Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Benincasa hispida</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215486			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The chemical was identified by accepted name [Di(2-ethylhexyl) phthalate (DEHP)].
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	A baseline value of no DEHP would be assumed, but no concurrent negative control was used.
	Metric 5:	Negative Control Response	Low	The biological response of a negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure 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	Details of exposure administration were reported, and exposures were administered consistently for the one exposure concentration.
	Metric 9:	Measurement of Test Substance Concentration	Low	Plant material was measured for DEHP, but not the air concentration.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was sufficient to detect plant uptake.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via fumigation.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source of the test plants was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test conditions were similar before and during the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Plant material was used as replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
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<b>Study Citation:</b>	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant <i>Benincasa hispida</i> and its use for lowering DEHP content of intercropped vegetables. <i>Journal of Agricultural and Food Chemistry</i> 61(22):5220-5225.
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Air; Dermal (topical application)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Benincasa hispida</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	ADME (biotransformation)
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2215486

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Low	Tissue measurement was adequate, but lack of air measurements or controls was concerning.
	Metric 18: Consistency of Outcome Assessment	Medium	There were minor differences in the timing of outcome assessment.
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis of uptake was unnecessary.
	Metric 22: Reporting of Data	Low	No control or baseline values were provided.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: An additional experiment assessed the decrease in air contaminated with DEHP with no mention of biological effects.			

**Overall Quality Determination****Low**

<b>Study Citation:</b>	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant <i>Benincasa hispida</i> and its use for lowering DEHP content of intercropped vegetables. <i>Journal of Agricultural and Food Chemistry</i> 61(22):5220-5225.			
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Air; Dermal (topical application)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Benincasa hispida</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	ADME (biotransformation)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2215486			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by accepted name [Di(2-ethylhexyl) phthalate (DEHP)].	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	High	Chemical purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	No concurrent negative control was used.	
	Metric 5: Negative Control Response	Low	The biological response of a negative control group was not reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure 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	Details of exposure administration were reported, and exposures were administered consistently for the one exposure concentration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Plant material was measured for DEHP, but not the air concentration.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was sufficient to detect plant uptake.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	Only one concentration was used.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via fumigation.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test plants was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Test conditions were similar before and during the exposure.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	Low	Tissue measurement was adequate, but lack of air measurements or controls was concerning.	
	Metric 18: Consistency of Outcome Assessment	Medium	There were minor differences in the timing of the outcome assessment.	

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<b>Study Citation:</b>	Wu, Z., Zhang, X., Wu, X., Shen, G., Du, Q., Mo, C. (2013). Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant <i>Benincasa hispida</i> and its use for lowering DEHP content of intercropped vegetables. <i>Journal of Agricultural and Food Chemistry</i> 61(22):5220-5225.		
<b>Duration:</b>	Overall Duration: > 21 days; Exposure Duration: > 21 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Air; Dermal (topical application)		
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Benincasa hispida</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	ADME (biotransformation)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2215486		
Domain	Metric	Rating	Comments
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis of uptake was unnecessary.
	Metric 22: Reporting of Data	Low	No control or baseline values were provided.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: An additional experiment assessed the decrease in air contaminated with DEHP with no mention of biological effects.			
<b>Overall Quality Determination</b>		<b>Low</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.	
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	MDA,"MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species."		

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8: Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9: Administration Measurement of Test Substance 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 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 Conditions	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 characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
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<b>Study Citation:</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	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**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Cucumis sativus* with DEHP exposure.

**Overall Quality Determination** **High**

<b>Study Citation:</b>	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cytotoxicity-Oxidative stress (including redox biology)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1987637			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	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 an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results	
	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 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/Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate for a dose response	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 and/or replicates was not reported, individual leaves comprised the replicates	
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	
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<b>Study Citation:</b>	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cytotoxicity-Oxidative stress (including redox biology)-Photosynthesis
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1987637

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group but n values were not reported
	Metric 23: Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and variability is reported. However, because it is unclear what constituted a technical replicate and how many replicates there were the reported SD is unclear.

Additional Comments: This includes ultra structural changes which were not quantifiably assessed or analyzed for statistical significance

## Overall Quality Determination

**Medium**

<b>Study Citation:</b>	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	1987637			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The chemical was identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Reporting omissions were unlikely to have a substantial impact on results.	
	Metric 8: Consistency of Exposure	High	Exposures were administered consistently across study groups.	
	Metric 9: Administration Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported (7 days) and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 and/or replicates was not reported, and individual leaves comprised the replicates.	
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	Zhang, Y., Wang, L., Du, N., Ma, G., Yang, A., Zhang, H., Wang, Z., Song, Q. (2014). Effects of diethylphthalate and di-(2-ethyl)hexylphthalate on the physiology and ultrastructure of cucumber seedlings. Environmental Science and Pollution Research 21(2):1020-1028.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Cucumis sativus</i> ; Jinchun; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	1987637

Domain	Metric	Rating	Comments
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	High	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented for each treatment and control group, but sample size and the numbers of technical replicates were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Medium	There were no unexpected outcomes, and a measure of variability was reported. However, it is unclear how variability was quantified (across plants, pots, how many individuals, etc).

Additional Comments: None

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: MDA”MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species.”			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8: Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9: Administration Measurement of Test Substance 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 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 Conditions	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 characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
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**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Lolium perenne</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Lolium perenne* with DEHP exposure.

**Overall Quality Determination** **High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance 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 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 Conditions	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 characterize 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
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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: MDA”MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species.”

## Overall Quality Determination

## High

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
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	Nothing was reported to indicate there were differences among groups that could influence the outcome assessment.	
Domain 7: Data Presentation and Analysis				
Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.	
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.	
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.	
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."			

**Overall Quality Determination****High**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Medicago sativa</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Medicago sativa* with DEHP exposure.

**Overall Quality Determination** **High**

<b>Study Citation:</b>	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. <i>Pedosphere</i> 27(6):1073-1082.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotiana tabacum</i> ; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5627041			
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 test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical was reported as "guaranteed reagent grade."
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.
	Metric 6:	Randomized Allocation	Medium	The study reported that test containers were randomly distributed.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare 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 an 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 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				
	Metric 13:	Test Organism Characteristics	Medium	The source of the seeds was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	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 plants was not reported. Three replicates were used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system (controlled chamber) were conducive to maintenance of organism health.
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<b>Study Citation:</b>	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. <i>Pedosphere</i> 27(6):1073-1082.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotiana tabacum</i> ; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	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 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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation is for germination effects.

## Overall Quality Determination

## High

<b>Study Citation:</b>	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. <i>Pedosphere</i> 27(6):1073-1082.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotiana tabacum</i> ; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5627041			
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 test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The chemical was reported as "guaranteed reagent grade."	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was reported and reasonable.	
	Metric 6: Randomized Allocation	Medium	The study reported that test containers were randomly distributed.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare 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 an 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 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				
	Metric 13: Test Organism Characteristics	Medium	The source of the seeds was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 plants was not reported. Three replicates were used.	
Domain 5: Outcome Assessment				
	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 Assessment	High	Outcomes were assessed consistently across study groups.	

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<b>Study Citation:</b>	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. <i>Pedosphere</i> 27(6):1073-1082.		
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotiana tabacum</i> ; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Development/Growth		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	5627041		
Domain	Metric	Rating	Comments
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.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were adequately described.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments: This evaluation is for vigor index and length.			
<b>Overall Quality Determination</b>		<b>High</b>	

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	792357			
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	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using 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 Characterization				
	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 the 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/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 and replicates were not reported.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tabacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	792357			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were 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 limited.
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	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.
<b>Additional Comments:</b>	This evaluation is for Growth (Development-Slowed, Retarded, Delayed or Non-development, Response Site: Not reported).			
<b>Overall Quality Determination</b>		<b>Low</b>		



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	792357			
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	The 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 Characterization				
	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 the 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/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 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 and replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	

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<b>Study Citation:</b>	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. <i>Allelopathy Journal</i> 27(1):87-96.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	792357

Domain	Metric	Rating	Comments
	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 limited.
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	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 evaluation is for germination.

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tabacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	792357			
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	The 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 Characterization				
	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 the 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/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 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 and replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	

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<b>Study Citation:</b>	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. <i>Allelopathy Journal</i> 27(1):87-96.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tobacum</i> ; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	792357

Domain	Metric	Rating	Comments
	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 limited.
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	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 evaluation is for Growth (Development-Slowed, Retarded, Delayed or Non-development, Response Site: Not reported).

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. <i>Allelopathy Journal</i> 27(1):87-96.			
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tabacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	792357			
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				
	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 Characterization				
	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 the 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/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 and replicates were not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.	

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<b>Study Citation:</b>	Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. <i>Allelopathy Journal</i> 27(1):87-96.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
<b>Exposure Route, Media, Path:</b>	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Nicotinana tabacum</i> ; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	792357

Domain	Metric	Rating	Comments
	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 limited.
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	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 evaluation is for germination.

## Overall Quality Determination

**Low**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test 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 influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: MDA”MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species.”				
<b>Overall Quality Determination</b>			<b>High</b>	



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8: Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9: Administration Measurement of Test Substance 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 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 Conditions	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 characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
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<b>Study Citation:</b>	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. <i>Frontiers of Environmental Science &amp; Engineering</i> 9(2):259-268.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	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**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Raphanus sativus</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Raphanus sativus* with DEHP exposure.

**Overall Quality Determination**

**High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
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 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 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	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 were 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 Conditions	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 characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test 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 influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group, and they were adequate to determine values for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	"DnBP and DEHP at a range of concentrations in the experimental soil showed no discernible effect on the germination rate of the seven test plant species. However, they did exert effects on root elongation, seedling growth and biomass to different extents, indicating the potential applicability of seedling growth in the evaluation of the phytotoxicity of PAE compounds."			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2915866			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	Source was reported, the test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.6%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported in Table 1 and 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 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 (168 hours of cultivation time for Allium Sepa and 72 hours for the other species).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Seeds exposed via soil.
Domain 4: Test 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 Conditions	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 characterize 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.

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<b>Study Citation:</b>	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.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2915866

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
	Metric 22: Reporting of Data	High	Percent germination was presented as a mean among the four treatment replicates for each treatment and control group were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This form represents the germination rate results presented in Table 1 for *Triticum aestivum* with DEHP exposure.

**Overall Quality Determination** **High**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was adequate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure 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 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 Conditions	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 characterize 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.	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups.	
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<b>Study Citation:</b>	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.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Embryo		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2915866		
Domain	Metric	Rating	Comments
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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Section 2.7 (page 3/10) does not present the statistical tests used to perform analysis other than the program used and p-value for significance.
Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments:	MDA, chlorophyll, carotenoid""MDA contents indicated distinctive resilience of ryegrass, alfalfa and onion under DnBP and DEHP stress compared with the other test species."		

**Overall Quality Determination****High**

<b>Study Citation:</b>	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Jingqiang 8; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493185			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was clearly identified. Chemical name and CASRN were provided. DEHP was purchased from Lark Technology Co., Ltd. (Beijing, China).The test substance identity was NOT analytically verified by the performing laboratory. Purity was 96.8%.
	Metric 2:	Test Substance Source	Low	
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Methanol was used as a solvent and a methanol control was used.
	Metric 5:	Negative Control Response	High	The biological responses of the control group were reported and normal.
	Metric 6:	Randomized Allocation	Medium	Seeds were placed at random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Soil was prepared by mixing such that final concentrations were 10, 20, and 40 mg/kg (dry weight). Methanol was evaporated. Concentrations were not verified
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure administration was reported, but because concentrations were not verified it is unclear how consistent exposures were across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	Low	The exposure duration was from seedling to booting stage of wheat , but it is unclear how long this is.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were mixed and estimated to be: 10, 20, and 40 mg/kg (dry weight).
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The source of the wheat seeds and rationale for selection of an agricultural crop species was described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Seeds were sterilized and washed prior to seeding and treatment environmental conditions were kept constant throughout the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 seeds were placed in each pot and 3 replicate pots were used per treatment group
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Jingqiang 8; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493185			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions and soil chemistry were reported and conducive to the health of the plants.	
	Metric 17: Outcome Assessment Methodology	Medium	The "mechanistic" endpoints examined included photosynthetic parameters and chlorophyll fluorescence, chlorophyll content in leaves, and biochemical activity of antioxidant enzymes SOD, CAT, MDA, GSH, and APX. The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.	
	Metric 18: Consistency of Outcome Assessment	Low	The measurements of biochemical enzymes were poorly described - authors state they followed kit protocols.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Background concentrations on contaminants in the soil were not measured. Environmental conditions of the treated plots over time were monitored but results were not reported.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest effects due to other variables, but background concentrations of contaminants in the soil were not measured.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Sufficient data are provided to conduct an statistical analysis if needed	
	Metric 22: Reporting of Data	High	Means and std error are reported for control and treatment groups	
	Metric 23: Explanation of Unexpected Outcomes	High	Damage caused by DEHP was mitigated by enzymatic activity in response to exposure.	
Additional Comments:	The authors exposed wheat to three concentrations of DEHP mixed with the soil, starting as seedlings and through to the reproductive stage. They measured kernal weight as well as various endpoints to quantify effects on photosynthesis, induction of reactive oxygen species, and response of the antioxidant defense system. Endpoints were examined at three stages: seedling, jointing, and booting. The exact exposure duration is unclear and concentrations were not measured			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Jingqiang 8; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493185			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The test substance was clearly identified. Chemical name and CASRN were provided.	
	Metric 2: Test Substance Source	Low	DEHP was purchased from Lark Technology Co., Ltd. (Beijing, China). The test substance identity was NOT analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Purity was 96.8%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Methanol was used as a solvent and a methanol control was used.	
	Metric 5: Negative Control Response	High	The biological responses of the control group were reported and normal.	
	Metric 6: Randomized Allocation	Medium	Seeds were placed at random.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Soil was prepared by mixing such that final concentrations were 10, 20, and 40 mg/kg (dry weight). Methanol was evaporated. Concentrations were not verified.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported, but because concentrations were not verified it is unclear how consistent exposures were across groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Low	The exposure duration was from seedling to booting stage of wheat, but it is unclear how long that was.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Exposure concentrations were mixed and estimated to be: 10, 20, and 40 mg/kg (dry weight).	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the wheat seeds and rationale for selection of an agricultural crop species was described.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Seeds were sterilized and washed prior to seeding, and treatment environmental conditions were kept constant throughout the experiment.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	Twenty seeds were placed in each pot and three replicate pots were used per treatment group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions and soil chemistry were reported and conducive to the health of the plants.	
	Metric 17: Outcome Assessment Methodology	Medium	The growth endpoint examined was the dry weight of the wheat kernel.	
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<b>Study Citation:</b>	Gao, M., Dong, Y., Liu, Y., Song, Z. (2018). Photosynthetic and antioxidant response of wheat to di(2-ethylhexyl) phthalate (DEHP) contamination in the soil. Chemosphere 209:258-267.			
<b>Duration:</b>	Overall Duration: Not-reported; Exposure Duration: Not-reported			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum aestivum</i> ; Jingqiang 8; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	5493185			
Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	It is unclear when the wheat kernels were collected and measured (after how many days of exposure for each treatment).
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Background concentrations on contaminants in the soil were not measured. Environmental conditions of the treated plots over time were monitored but results were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest effects due to other variables, but background concentrations of contaminants in the soil were not measured.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Sufficient data are provided to conduct a statistical analysis if needed.
	Metric 22:	Reporting of Data	High	Means and standard error were reported for control and treatment groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	Damage caused by DEHP was mitigated by enzymatic activity in response to exposure.
<b>Additional Comments:</b>	The authors exposed wheat to three concentrations of DEHP mixed with the soil, starting as seedlings and through to the reproductive stage. They measured kernel weight as well as various endpoints to quantify effects on photosynthesis, induction of reactive oxygen species, and response of the antioxidant defense system. Endpoints were examined at three stages: seedling, jointing, and booting. The exact exposure duration is unclear and concentrations were not measured.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3350318			
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	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Chemical purity reported as 96.8%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.	
	Metric 5: Negative Control Response	High	The biological response of the negative control group was suitable.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. Test solutions were replenished daily.	
	Metric 8: Consistency of Exposure 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 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 biological response of the solvent control was acceptable.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	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 ( 8 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 Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	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 ± 1oC and 12-h nights at 20 ± 1 oC at 60% relative humidity level.”
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodologies for plant height, fresh weights of shoots and roots and dry weight of shoots and roots were reported but not in sufficient detail.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported, but these are unlikely to have a substantial impact on results. The experiments were conducted in an artificial climate chamber.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	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 multiple 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 discussion of the effects of DBP and DEHP on growth indices of wheat seedlings was not clear, and their conclusions were made without incorporating any discussion of statistical significance. Results of multiple comparisons were not provided.			

**Overall Quality Determination****Medium**



<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3350318			
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	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 96.8%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail. Test solutions were replenished daily.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance 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	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 biological response of the solvent control was acceptable.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	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 (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 Assessment				
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<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Root uptake			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Triticum sp</i> ; Jinnong 7; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Photosynthesis			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	3350318			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health. The experiments were conducted in an artificial climate chamber. The seedlings were cultured under a cycle of 12-h days at 25C and 12-h nights at 20C at 60% relative humidity level.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodologies for chlorophyll content, photosynthetic parameters, and chlorophyll fluorescence were reported in detail.
	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	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 a controlled environment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	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 multiple comparisons were not provided.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	The author's discussion of the effects of DBP and DEHP on growth indices and mechanistic endpoints of wheat seedlings was not clear, and their conclusions were made without incorporating any discussion of statistical significance. Results of multiple comparisons were not provided.			

**Overall Quality Determination****High**

<b>Study Citation:</b>	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. <i>Pedosphere</i> 24(1):107-115.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2510954			
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				
	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 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	Medium	exposures were administered consistently across study groups, albeit with few details
	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 adequate for a dose response
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment Conditions	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 characterize 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
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<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2510954			
Domain	Metric	Rating	Comments	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test 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 Presentation and Analysis				
	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.			
<b>Overall Quality Determination</b>		<b>Medium</b>		

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<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2510954			
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 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	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	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures were administered consistently across study groups, albeit with few details.	
	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 adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	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 characterize toxicological effects.	
Domain 5: Outcome Assessment				
	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	High	Outcomes were assessed consistently across study groups.	
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<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Reproductive/Teratogenic
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)
<b>HERO ID:</b>	2510954

Domain	Metric	Rating	Comments
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 nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: This form is to assess the germination outcome.			

**Overall Quality Determination****Medium**

<b>Study Citation:</b>	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.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)			
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)			
<b>HERO ID:</b>	2510954			
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				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported and adequate	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups	
Domain 3: Exposure 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	Medium	exposures were administered consistently across study groups, albeit with few details	
	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	
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	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source	
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	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize 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	
	Metric 18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups	
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<b>Study Citation:</b>	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. <i>Pedosphere</i> 24(1):107-115.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vegetation; Vascular Plants; <i>Vigna radiata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)		
<b>Chemical:</b>	Di-ethylhexyl phthalate (DEHP)		
<b>HERO ID:</b>	2510954		
Domain	Metric	Rating	Comments
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	Nothing reported to indicate there were differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were adequately described
Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes
Additional Comments:	Mechanistic endpoints are: malondialdehyde (MDA) Polyphenol oxidase (PPO)ascorbate peroxidase (APX)Superoxide dismutase (SOF)The glutathione (GSH)peroxidase (POD)Proline content		
<b>Overall Quality Determination</b>		<b>High</b>	