



Data Quality Evaluation Information for Environmental Hazard for Di-isobutyl Phthalate (DIBP) (1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 84-69-5

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July 2025

This supplemental file contains information regarding the data quality evaluation results relevant to the analysis of environmental hazard for the *Draft Environmental Hazard Assessment for Diisobutyl Phthalate (DIBP)*. Due to data gaps identified for DIBP, a read-across analog approach was employed and DBP data were used to fill data gaps in the *Draft Environmental Hazard Assessment for Diisobutyl Phthalate (DIBP)*. 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 Diisobutyl Phthalate (DIBP) - Systematic Review Protocol*.

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

Diisobutyl Phthalate Table of Contents

Lasius niger

Table of Contents

HERO ID Reference Page **Diisobutyl Phthalate Habitat: Aquatic (freshwater)** Taxa: Vertebrates Pimephales promelas 11581733 Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) - The potential application of omics data in risk evaluations under TSCA (internal use only). **Habitat: Aquatic (marine)** Taxa: Plants (Non-vascular) Karenia brevis 3230225 Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 20 155:498-508. **Habitat: Aquatic (brackish) Taxa: Invertebrates** Nitocra spinipes 51937 Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against 23 two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851. **Habitat: Terrestrial Taxa: Invertebrates** Caenorhabditis elegans 2215375 25 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.

Page **3** of **166**

33

35

53

55

59

Diisobutyl Phthalate	Table of Contents
2347468	Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution.

Taxa: Plants (Vascular)

Nicotiana tabacum

5627041 Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on

seed germination and seedling growth. Pedosphere 27(6):1073-1082.

Environmental Science and Pollution Research 21(23):13446-13451.

Nicotinana tobacum

792357 Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy

Journal 27(1):87-96.

Analog Chemical Data

Cyprinodon variegatus

Habitat: Aquatic (freshwater)

Taxa: Vertebrates

1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to	47
1321990	Adams, W. J., Biddinger, G. R., Robinard, R. A., Gorsden, J. W. (1993). A summary of the acute toxicity of 14 philiatate esters to	4/
	representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	

Danio rerio

1316201

2298079	Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting	49
	activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.	

Lepomis macrochirus

1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to	51
	representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574	

Bionomics, EG&G (1983). Exhibit III: Acute toxicity of thirteen phthalate esters to bluegill (Lepomis macrochirus).

18064 Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bulletin of

Environmental Contamination and Toxicology 26(4):446-452.

Oncorhynchus mykiss

EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-

through conditions.

6571362 EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-

through conditions.

Oncorhynchus mykiss (Salmo gairdneri)

Diisobuty	yl Phthalate	Table of Contents	
5530771 Bionomics,, EG&G (1983). Acute toxicity of f (final report) report no BW-83-3-1373.		Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report) report no BW-83-3-1373.	63
	Oryzias latipes		
10064186		EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).	65
10064186		EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).	69
	Oryzias melastigma		
2298079		Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.	85
	Pimephales promelas		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	87
1336024		Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.	91
1336024		Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.	93
	Salmo mykiss		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	95
Т	Taxa: Invertebrates		
	Chironomus plumosus		
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	97
1332972		Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).	101
	Chironomus tentans		
679311		Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.	103
	Daphnia magna		
1321996		Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	107
1336024		Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental Toxicology and Chemistry 4(2):167-179.	109

Page **5** of **166**

Diisobutyl I	Phthalate Table of Contents	
4829279	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBI to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.	111
1336024	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow Environmental Toxicology and Chemistry 4(2):167-179.	113
4829279	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBI to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.	119
	Hyalella azteca	
679311	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W. Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.	
	Paratanytarsus parthenogenetica	
1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	127
	Paratanytarsus parthenogenica	
1316219	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424.	129
Tax	xa: Plants (Non-vascular)	
	Selenastrum capricornutum	
1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	131
Habit	eat: Aquatic (marine)	
Tax	xa: Invertebrates	
	Animalia	
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	133
	Annelida	
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	137
	Arthropoda	
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	141

Chordata

Diisobutyl Phthalate	Table of Contents	
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	145
Coelenterata		
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	149
Echinodermata		
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	153
Mollusca		
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	157
Mysidopsis bahia		
1321996	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.	161
Rhynchocoela		
5495608	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.	163
Habitat: Aquatic (brackish)	
Taxa: Invertebrate	rs ·	
Nitocra spinipes		

Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against

two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.

165

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Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Vertebrate; Fish; Pimephales promelas; Larvae Taxa, Species, Age:

Health Outcome: Behavioral

Chemical: Diisobutyl Phthalate- Parent compound

Domain	Metric	Rating	Comments		
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.		
Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no information was given on analytical verification.		
Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.		
Domain 2: Test Design					
Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.		
Metric 5:	Negative Control Response	Low	The goal of the behavior analysis was to calculate a behavior-based point of departure. While the authors reported excluding any inactivity across treatment groups from analysis, the authors did not specifically report if the control group behaved as expected.		
Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to chemical exposure beakers and the beakers were randomly arranged on trays in the incubator. In addition, for the behavior analysis to control for positional effects, fish from each of the eight exposure conditions were loaded in order into each of the 6 wells of the first row, with the remaining two being placed in the first two positions of the second row; this pattern was subsequently repeated across replicate plates.		
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. After the 24 h exposure live larvae from each exposure vessel were transferred into small plastic weight boats for the behavioral analysis.		
Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.		
Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC-MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).		
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours followed by a behavioral assessment that lasted 28 minutes. This was an adequate exposure time to capture dose-response effects and an adequate behavior assay duration to capture changes in movement patterns.		

Diisobutyl Phthalate Environmental Hazard Evaluation

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Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 11581733 Table: 1 of 4

Media, Path: Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome:

Behavioral

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 11581733

Domain	Metric	Rating	Comments
Metric 11	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups was adequate (11 treatment groups). Specific for the behavior assay, authors collected movement data for the control, control + O-ring, and the 6 highest treatment groups without significant mortality. The authors did not show movement data across treatment groups; instead, they used the data to calculate the behavior point of departure.
Metric 12	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical-saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.
Domain 4: Test Organism			
Metric 13	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.
Metric 14	Acclimatization and Pretreatment Conditions	Medium	Authors did not specifically mention acclimating test organisms prior to the exposure start but culture conditions for all larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water). Moreover, for the behavior assay, fish were acclimated for 10 min in the dark.
Metric 15	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the chemical exposure. For the behavioral analyses, 3 larvae were used per replicate.
Domain 5: Outcome Assessment			
Metric 16	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the approved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
Metric 17	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure vessel were transferred into well plates for the behavior analysis. The behavior analysis was described in detail on page 8 and 13.
Metric 18	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.

Domain 6: Confounding / Variable Control

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Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 11581733 Table: 1 of 4

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Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E. H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential application of omics data in risk evaluations under TSCA (internal use only).

Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome: Behavioral

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 11581733

Domain		Metric	Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout the experiment. For the behavior assay, fish across treatment groups were handled in the same manner (e.g., transfer from beakers to well-plates, acclimation period in well-plates). While authors did not specifically mention acclimating test organisms prior to the chemical exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	The behavioral data was analyzed using the ZebraLab software. The bPOD calculation was described in detail on page 14.
	Metric 22:	Reporting of Data	Medium	Calculation of all points of departure measurements was described in the text (methods and results) and Table 6 shows the bevavior-based POD value for DBP. However, actual behavior data (movement patterns) across treatment groups were not included in the report.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not given with the behavioral data.
Additional Comments:		goal of this study was to investigate the po		eation of omics data in risk evaluation. This data evaluation is for the calculation of the g exposure to DIBP.

Overall Quality Determination

Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Evnocure Doute	A quetia (freehweter): Weter: Not determined by study outhors (i.e., shemical of interact in exposure water, but unable to determine exect untake route)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: **Health Outcome:**

Vertebrate; Fish; Pimephales promelas; Larvae

Mechanistic-Cell signaling/function Diisobutyl Phthalate- Parent compound **Chemical:**

HERO ID: 11581733

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.
	Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no information was given on analytical verification.
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.
	Metric 5:	Negative Control Response	Medium	The effect measured is gene expression (transcriptomic analysis). There is no gene expression profile established for control/unexposed organisms in this developmental stage for this species, particularly gene expression data from the entire fish. Also, given the nature of the collection of fertilized eggs and an outbred population, gene expression even among unexposed fish is expected to vary. From a sequencing perspective, there were no unexpected results.
	Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trays in the incubator.
Domain 3: Exposure C	haracterization			
Zomani or Ziiposai e e	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. The RNA analysis was described on pages 8-10.
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC-MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe transcriptomic changes.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups (11 treatment groups) and spacing were adequate for the transcriptomics analysis and POD derivation.
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical-saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.

Domain 4: Test Organism

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	1 19
Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) - The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route.	Aquatic (freshwater): Water: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Exposure Rou Media, Path:

Taxa, Species, Age: **Health Outcome:**

Vertebrate; Fish; Pimephales promelas; Larvae

Chemical: **HERO ID:**

Mechanistic-Cell signaling/function Diisobutyl Phthalate- Parent compound

11581733

HERO ID:	11361/33			
Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors didn't specifically mention acclimating test organisms prior to the exposure start but culture conditions for the larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the exposure. For the RNA analysis there were 4 larvae per replicate. Each individual well contained one larva.
Domain 5: Outcome	Assessment			
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the approved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure vessel were used for RNA analysis. The sample processing, RNA isolation, RNA sequencing, and tPOD calculation were described in detail on pages 8-10.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.
Domain 6: Confound	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	tPOD calculation was described on pages 10 and 11 and seemed appropriate to assess results.
	Metric 22:	Reporting of Data	High	Table 4 shows tPOD data and some results are described in the "PODs" section of the

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 11581733 Table: 2 of 4

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Study Citation: Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.

H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential

application of omics data in risk evaluations under TSCA (internal use only).

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Vertebrate; Fish; *Pimephales promelas*; Larvae

Health Outcome: Mechanistic-Cell signaling/function
Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 11581733

Domain		Metric	Rating	Comments	
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability were given with the tPOD data.	

Additional Comments: The primary goal of this study was to investigate the potential application of omics data in risk evaluation. This data evaluation is for the calculation of the transcriptomics-based point of departure following exposure DIBP.

Overall Quality Determination

Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Ermoguno Douto	A quatic (freehyystem). Water Not determined by study outhors (i.e., showing) of interest in averagement water but unable to determine average untaken moute)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Health Outcome: Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome: Chemical: Mechanistic-Cell signaling/function Diisobutyl Phthalate- Parent compound

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.
Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no information was given on analytical verification.
Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.
Metric 5:	Negative Control Response	High	The effect measured is metabolomics. There is no metabolomic profile established for control/unexposed organisms in this developmental stage for this species, particularly a metabolomics profile from the entire fish. That said, to best evaluate 'normality' of the control response, the metabolite profiles of the fathead minoow larvae with and without o-rings (i.e. control vs. vehicle control) were compared and no discernable differences in their profiles were observed leading to the conclusion of 'no unexpected observed measurements in controls'. Also, given the nature of the collection of fertilized eggs and an outbred population, a metabolomic profile even among unexposed fish is expected to vary. From a spectra analysis perspective, there were no unexpected results.
Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trays in the incubator.
Domain 3: Exposure Characterization	1		
Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7. The metabolomics analysis procedure was further described on page 11.
Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.
Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC-MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe metabolomic results.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups (11 treatment groups) and spacing were adequate to obtain changes in metabolomics data.

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Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 11581733 Table: 3 of 4

Taxa, Species, Age: Health Outcome: Vertebrate; Fish; Pimephales promelas; Larvae

Chemical:

Mechanistic-Cell signaling/function Diisobutyl Phthalate- Parent compound

HERO ID:	11581733	ntharate- Parent compound		
Domain		Metric	Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical-saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.
Domain 4: Test Organ	nism			
_	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors didn't specifically mention acclimating test organisms prior to the exposure start but culture conditions for the larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups for the exposure. For the metabolomics analysis there were 3 larvae per replicate. Three larvae from each set of the replicate treatments were transferred to individual wells of a 1.0 mL 96-well deep well plate.
Domain 5: Outcome	Assessment			
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the approved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis. Live larvae from each exposure vessel were used for metabolomics analysis. The metabolite extraction, metabolite derivatization, GC-qToF/MS analysis, and mPOD calculation were described in detail on pages 10-12.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.
Domain 6: Confound	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 11581733 Table: 3 of 4

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Study Citation: Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.

H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) - The potential

application of omics data in risk evaluations under TSCA (internal use only).

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

 Taxa, Species, Age:
 Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome: Mechanistic-Cell signaling/function
Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 11581733

Domain		Metric	Rating	Comments
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.
Domain 7: Data Pres	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Metabolomics data analysis and mPOD calculation was described on pages 11 and 12 and seemed appropriate to assess results.
	Metric 22:	Reporting of Data	High	Table 5 shows mPOD data and some results are described in the "PODs" section of the results.
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability were given with the mPOD data.

metabole

The primary goal of this study was to investigate the potential application of omics data in risk evaluation. This data evaluation is for the calculation of the metabolomics-based point of departure following exposure to DiBP.

Overall Quality Determination

Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substan				
	Metric 1:	Test Substance Identity	High	The chemical name and CASRN were reported.
	Metric 2:	Test Substance Source	Low	The test substance was purchased from MilliporeSigma (Burlington, MA), but no information was given on analytical verification.
	Metric 3:	Test Substance Purity	High	The purity was reported as greater than or equal to 98%.
Domain 2: Test Design				
Č	Metric 4:	Negative Controls	High	A control and a control plus O-ring group were tested.
	Metric 5:	Negative Control Response	High	There was 0% total mortality in the control test groups.
	Metric 6:	Randomized Allocation	Medium	Larvae were randomly distributed to exposure beakers and the beakers were randomly arranged on trays in the incubator.
Domain 3: Exposure Ch	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was described in detail on page 6 and 7. A passive dosing design using O-rings was used due to the low water solubility of the test substance. The exposure system was adequately described on page 7.
	Metric 8:	Consistency of Exposure	High	Exposures were run consistently across treatment groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Measurements of the test substance water concentrations were carried out using an LC-MS system. Measurements were taken in the stock solutions at time zero and at the completion of exposure (24 hours).
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was 24 hours and was an appropriate time length to observe mortality effects.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups was adequate for the experimental design (11 treatment groups), and spacing was appropriate as a NOEC was determined at the end of the study (i.e., lowest concentration was low enough).
	Metric 12:	Testing at or Below Solubility Limit	High	Due to the low solubility of the test substance, a passive dosing design using a chemical saturated high purity silicone O-ring was used, along with diluting stock solutions in methanol.
Domain 4: Test Organis	m			
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	High	FHM larvae were obtained from an on-site breeding culture at AWBERC in Cincinnati, OH. Test organisms were described on pages 4 and 5.

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Study Citation:	Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.
	H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential
	application of omics data in risk evaluations under TSCA (internal use only).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Larvae

Health Outcome:

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID:	11581/33

HERO ID:	11581733			
Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Authors did not specifically mention acclimating test organisms prior to the exposure start but culture conditions for all larvae were similar to exposure conditions (e.g., 25 degree C incubator, source of water +/- phthalate).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 13 larvae placed in each treatment beaker and there were 3 replicates of each of the 11 treatment groups.
Domain 5: Outcome Ass	sessment			
Domain 3. Outcome 1180	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate. Exposures were conducted in accordance with the approved AWBERC Animal Care and Use Protocol. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 17:	Outcome Assessment Methodology	High	Following the 24-hour exposure, mortality was recorded in each beaker and dead larvae were removed. High concentrations with substantial (> 10%) mortality were excluded from further analysis.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across treatment groups.
Domain 6: Confounding			TT' 1	
	Metric 19:	Confounding Variables in Test Design and Procedures	High	All study treatment groups were treated equally throughout experiment. While authors did not specifically mention acclimating test organisms prior to the exposure start, culture conditions and exposure conditions (e.g., minus absence/presence chemical) were similar and appropriate pre-exposure and during exposure. Routine water chemistries were within normal ranges and both temperature and pH levels exhibited little change throughout the experiment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information given about differences among treatment group organisms that could influence the outcome assessment.
D : 7 D : D :	.· 1 A 1			
Domain 7: Data Presenta		-	TT: _1.	LOSO 1 14' 1 1' 14' 14' 14 1 1 1 1 NOTO
	Metric 21:	Statistical Methods	High	LC50 calculation was described in detail in the supplental document. Also, NOEC derivation was briefly described on page 13 of the report, and Table 2 reported the raw data and percent mortality.
	Metric 22:	Reporting of Data	High	Table 2 (2C) showed the mortality data across all treatments for all 3 replicates.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not given with the mortality data.
Additional Comments:	The primary with DiBP.	goal of this study was to investigate the po	otential appli	cation of omics data in risk evaluation. This evaluation is for the mortality assessmen
		Conti	nued on nex	at page

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Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 11581733 Table: 4 of 4

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Study Citation: Bencic, D. C., Flick, R. W., Bell, M. E., Henderson, W. M., Huang, W., Purucker, S. T., Glinski, D. A., Blackwell, B. R., Christen, C. H., Stacy, E.

H., Biales, A. D. (2024). A multiomics study following acute exposures to phthalates in larval fathead minnows (Pimephales promelas) – The potential

application of omics data in risk evaluations under TSCA (internal use only).

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Pimephales promelas*; Larvae

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 11581733

Domain Metric Rating Comments

Overall Quality Determination High

Study Citation:	Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Aquatic (marine); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake

Media, Path:

Vegetation; Non-vascular Plants; Karenia brevis; Not Applicable (e.g., fungi or algae studies) or Not Reported Taxa, Species, Age:

Health Outcome: Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	Low	Relevant test materials are described as di-iso-butyl ortho-phthalate (DIBP), benzyl-n-butyl ortho-phthalate (BBP), Di-n-butylortho-phthalate (DBP), and bis(2-ethylhexyl)ortho-phthalate(DEHP). No further details are provided.
	Metric 2:	Test Substance Source	Low	Chemicals are sourced by the Sigma Company, no additional information provided.
	Metric 3:	Test Substance Purity	High	Purity is reported as > 99%.
Domain 2: Test Design				
2011am 2, 1000 2 co.g.	Metric 4:	Negative Controls	High	Negative and solvent controls were used. The authors indicated that no significant differences were observed between the solvent controls (acetone concentration (0.5 mL L-1)) and the negative control.
	Metric 5:	Negative Control Response	High	No adverse effects reported in the control.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	araatarization			
Domain 3. Exposure Cii	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and minimize loss of test substance before and during the exposure for these degradable substances. A solvent (acetone) was used to facilitate the 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 is likely to be observed, the reported nominal concentrations are not likely to be representative of the final concentration, and reporting in terms of nominal concentrations may underestimate the effects observed.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type and/or outcome(s) of interest (96-hour algae growth inhibition test).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure concentrations were reported to be 0, 1, 5, 10, 20, 30, 50, 100, 150, 200 mL/L. These appear to reflect the nominal concentration and no final test quantification was conducted, so these are likely not representative of the actual exposure concentration throughout the test. The number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.

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Study Citation: Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508. **Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Aquatic (marine); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake

Media, Path:

Vegetation; Non-vascular Plants; Karenia brevis; Not Applicable (e.g., fungi or algae studies) or Not Reported

Taxa, Species, Age: **Health Outcome:**

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID:	3230225			
Domain		Metric	Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate (the authors demonstrated that no significant difference (p > 0.05) was observed between the growth in controls and acetone treatment.).
Domain 4: Test Organi	sm			
C	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 organisms (algal density) was not reported.
Domain 5: Outcome A	ssessment			
	Metric 16:	Adequacy of Test Conditions	High	The algal cells were cultured in a GXZ-380Z intelligent illumination incubator under the following conditions: 14 h of light at 24C (4000 ± 500 lux) and 10 h of dark at 22C. The medium was shaken once daily to prevent cell adherence growth.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported; it was unclear whether methods were sensitive for the outcome of interest. The authors reported that significant effects were observed on growth following exposure to some of the test materials, but did not provide the measures of significance for each test concentration, so it was not clear what levels elicited these inhibitions on growth. There appeared to be a doseresponse based on the graphs provided. This is likely to have a substantial impact on results.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments appear to be consistent across groups. Cell numbers were determined every 24 hours.
Domain 6: Confoundin	o / Variable Co	ntrol		
Domain of Companion	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 Preser	tation and Anal	vsis		
	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. Details of EC50 calculations were not provided.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 3230225 Table: 1 of 1

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Study Citation: Liu, N., Wen, F., Li, F., Zheng, X., Liang, Z., Zheng, H. (2016). Inhibitory mechanism of phthalate esters on Karenia brevis. Chemosphere 155:498-508.

Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Aquatic (marine); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake

Media, Path: route

Vegetation; Non-vascular Plants; *Karenia brevis*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Taxa, Species, Age: Health Outcome:

Development/Growth

Chemical: Development Disso

Diisobutyl Phthalate- Parent compound

HERO ID: 3230225

Domain		Metric	Rating	Comments
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were shown for each treatment and control group (Figure 1). The author's discussion of the growth inhibition observed for DIBP was not clear, and their conclusions were made without incorporating any discussion of statistical significance.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, or unexpected outcomes were satisfactorily explained.

Additional Comments: The discussion of growth inhibition following exposure to DEHP, DIBP, BBP and DBP was lacking.

Overall Quality Determination

Low

Study Citation:	Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979)	. The acute toxicity of 78 chemicals and pestici	de formulations against two brackish
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water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Nitocra spinipes; Adult

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 51937

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization			
•	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure and exposure frequency were reported and suitable, but slightly longer than typical for the study type.
	Metric 11:	Number of Exposure Groups/	Medium	At least six concentrations were tested, but a range of levels was not reported.
	Metric 12:	Spacing of Exposure Levels Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. However the reported LC50 (3.0 mg/L) was below the water solubility given in the Final Scope for DIBP, 6.2 mg/L at 25C.
Domain 4: Test Organis	m			
C	Metric 13:	Test Organism Characteristics	Medium	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It is unclear if test organisms were acclimatized to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 20 organisms with no replicates used per treatment.

Domain 5: Outcome Assessment

Continued on next page ...

HERO ID: 51937 Table: 1 of 1

Diisobutyl Phthalate Environmental Hazard Evaluation

... continued from previous page

Study Citation: Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish

water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Nitocra spinipes; Adult

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 51937

Domain	Metric	Rating	Comments
Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and seemed consistent.
Domain 6: Confounding / Variable Co	ontrol		
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Ana	lysis		
Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the table.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and
	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome: Behavioral

Chemical: Diisobutyl Phthalate- Parent compound

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 Char	acterization			
_	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
]	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
]	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured
]	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported and adequate for the study type
]	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were adequate for a dose
		Spacing of Exposure Levels		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	High	all pretreatment conditions were the same for control and exposed organisms
1	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)
Domain 5: Outcome Asses	ssment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2215375 Table: 1 of 4

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Study Citation: Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and

AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome: Behavioral

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 2215375

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups
		Assessment	-	
Domain 6: Confound	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: locomotor and thermotaxis

Overall Quality Determination

Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and
	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.
Duration:	Overall Duration: 0 - 4 days (0-96h): Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome:

Mechanistic-Oxidative stress (including redox biology)

Chemical:

Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name
	Metric 2:	Test Substance Source	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 Cl	haracterization			
•	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was reported and adequate for the study type, but there was no "No Effect level"
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and spacing of exposure levels were adequate for a dose response but there was no "No Effect level"
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium
Domain 4: Test Organis	em.			
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	all pretreatment conditions were the same for control and exposed organisms
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)
Damain & Outra				
Domain 5: Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2215375 Table: 2 of 4

... continued from previous page

Study Citation: Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and

AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome: Mechanistic-Oxidative stress (including redox biology)

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 2215375

Domain		Metric	Rating	Comments
Domain 6: Confound	ing / Variable Co	atral		
Domain o. Comound	C			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Preso	entation and Anal Metric 21:	ysis Statistical Methods	High	Statistical methods were adequately described
	Metric 21: Metric 22:	Statistical Methods Reporting of Data	High 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 Commant	a. Ethanal must	mantment magative avvisor amazina		
Additional Comment	s: Emanoi pren	reatment, reactive oxygen species		

Overall Quality Determination

Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and
	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome: Mechanistic-Oxidative stress (including redox biology)

Chemical: Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name
	Metric 2:	Test Substance Source	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 C	naracterization			
2 _F 23410 O.	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Concentration Exposure Duration and Frequency	Low	The duration of exposure was reported and adequate for the study type, but there was no "No Effect level"
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and spacing of exposure levels were adequate for a dose response but there was no "No Effect level"
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via culture medium
D : 1 T : 0 :				
Domain 4: Test Organis	m Metric 13:	Test Ousenism Characteristics	High	
		Test Organism Characteristics Acclimatization and Pretreatment	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:		High	all pretreatment conditions were the same for control and exposed organisms
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test organisms was not reported, repeated tests were used as replicates (n=3)
		respicates per Group		
Domain 5: Outcome As	ssessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2215375 Table: 3 of 4

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Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and

AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; wild type; Larvae

Health Outcome: Mechanistic-Oxidative stress (including redox biology)

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 2215375

Domain		Metric	Rating	Comments
Domain 6: Confoundi	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups that could influence the outcome assessment.
		1	Iligii	uncre were no unreferrees among groups that could influence the outcome assessment.
D : 7 D : D		1	High	uncre were no unreferrees among groups that could influence the outcome assessment.
Domain 7: Data Prese	entation and Anal Metric 21:	ysis Statistical Methods	High	Statistical methods were adequately described
Domain 7: Data Prese	entation and Anal	ysis		

Overall Quality Determination

Study Citation:	Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and
	AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; DA 1267; Larvae

Health Outcome:

Mechanistic-Neurotoxicology-Ocular and Sensory

Chemical:

Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was adequate.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	naracterization			
*	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	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.
Damain 4. Tast Organia				
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 13:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	MICHIC 14.	Conditions	High	An preneament 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 As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2215375 Table: 4 of 4

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Study Citation: Tseng, I. L., Yang, Y. F., Yu, C. W., Li, W. H., Liao, C., V.H. (2013). Phthalates induce neurotoxicity affection	g locomotor and thermotactic behaviors and
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AFD neurons through oxidative stress in Caenorhabditis elegans. PLoS ONE 8(12):e82657.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Caenorhabditis elegans; DA 1267; Larvae

Health Outcome: Mechanistic-Neurotoxicology-Ocular and Sensory Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 2215375

Domain		Metric	Rating	Comments
Domain 6: Confound	ng / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups that could influence the outcome assessment.
D : 7 D : D		•	Tilgii	There were no differences among groups that could influence the outcome assessment.
	Wictite 20.	Outcomes Officiated to Exposure	Tilgii	There were no differences among groups that could influence the outcome assessment.
Domain 7: Data Prese	entation and Anal Metric 21:	ysis Statistical Methods	High	Statistical methods were adequately described.
Domain 7: Data Prese	entation and Anal	ysis		

Overall Quality Determination

Study Citation: Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. Environmental

Science and Pollution Research 21(23):13446-13451.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days **Exposure Route,** Terrestrial; N/A (e.g., injection); Dermal (topical application)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Lasius niger; Adult

Health Outcome: ADME (biotransformation)

Chemical: Diisobutyl Phthalate- Parent compound

TERU ID:	2347408			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical was 99% pure.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent solvent control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was suitable.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	haracterization			
•	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	Medium	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were measured after administration and presented as time 0
	wietrie 7.	Concentration	Low	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 fo the study type.
	Metric 11:	Number of Exposure Groups/	N/A	Only one dose was used.
		Spacing of Exposure Levels		
	Metric 12:	Testing at or Below Solubility Limit	N/A	The test substance was solubilized in methanol prior to topical application.
Domain 4: Test Organi	sm			
· ·	Metric 13:	Test Organism Characteristics	Medium	There are minor reservations about source of the test organisms.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
	35.1.15	Conditions	3.6.11	
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to characte
		Replicates per Group		ize toxicological effects.
Domain 5: Outcome A	ssessment			
	Metric 16:	Adequacy of Test Conditions	Low	Minor uncertainties were identified regarding environmental conditions of the test system due to few details reported.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2347468 Table: 1 of 1

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Study Citation: Lenoir, A., Touchard, A., Devers, S., Christidès, J. P., Boulay, R., Cuvillier-Hot, V. (2014). Ant cuticular response to phthalate pollution. Environmental

Science and Pollution Research 21(23):13446-13451.

Duration: Exposure Route, Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days Terrestrial; N/A (e.g., injection); Dermal (topical application)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; *Lasius niger*; Adult

Health Outcome:

ADME (biotransformation)

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 2347468

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcomes of interest with some uncertainty.
	Metric 18:	Consistency of Outcome Assessment	Medium	There was incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no differences among groups, but there were few details to confirm that.
Domain 7: Data Pres	sentation and Anal	lysis		
	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

Study Citation:	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination
	and seedling growth. Pedosphere 27(6):1073-1082.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route,	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Modio Poth	

Media, Path:

Vegetation; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported

Taxa, Species, Age: **Health Outcome:**

Reproductive/Teratogenic

Chemical:

Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substa				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The test substance was reported as "guaranteed reagent grade."
Domain 2: Test Design	1			
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.
	Metric 6:	Randomized Allocation	Medium	The study reported that test containers were randomly distributed.
Domain 3: Exposure C	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 assessment was difficult to determine.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/ 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 Organ	ism			
	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, but three replicates were used.
Domain 5: Outcome A	ssessment			
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system (controlled chamber) were conducive to maintenance of organism health.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5627041 Table: 1 of 2

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Study Citation:	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination
	and seedling growth. Pedosphere 27(6):1073-1082

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vegetation; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Reproductive/Teratogenic

Chemical: Diisobut: **HERO ID:** 5627041

Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment		
Domain 6: Confound	ling / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	veis		
Domain 7. Data 110s	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This form is for germination effects.

Overall Quality Determination

Study Citation:	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination
	and seedling growth. Pedosphere 27(6):1073-1082.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Media, Path:

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vegetation; Vascular Plants; Nicotiana tabacum; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

neko id:	3027041			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The test substance was reported as "guaranteed reagent grade."
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.
	Metric 6:	Randomized Allocation	Medium	The study reported that test containers were randomly distributed.
Domain 3: Exposure Ch	naracterization			
1	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 assessment was difficult to determine.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	metric 11.	Spacing of Exposure Levels	ing.	response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	m			
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Medium	The source of the seeds was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of test plants was not reported, but three replicates were used.
Domain 5: Outcome As	gaggmant			
Domain 5. Outcome As	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.
			tinued on nex	of page
		Con		L

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5627041 Table: 2 of 2

... continued from previous page

Study Citation:	Deng, J., Zhang, Y., Hu, J., Jiao, J., Hu, F., Li, H., Zhang, S. (2017). Autotoxicity of phthalate esters in tobacco root exudates: Effects on seed germination	
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and seedling growth. Pedosphere 27(6):1073-1082.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vegetation; Vascular Plants; *Nicotiana tabacum*; cv K326; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 5627041

Domain		Metric	Rating	Comments
Domain 6: Confound	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
			_	Unexpected outcomes were satisfactorily explained.

Overall Quality Determination

96. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days **Duration:**

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route,**

Media, Path:

Vegetation; Vascular Plants; Nicotinana tobacum; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported Taxa, Species, Age:

Reproductive/Teratogenic **Health Outcome:**

Diisobutyl Phthalate- Parent compound Chemical:

HERO ID: 792357

Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.
Domain 2: Test Design	1			
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.
Domain 3: Exposure C	haracterization			
Domain 3. Exposure C	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (6.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.
Domain 4: Test Organi	ism			
Ü	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether pretreatment conditions were the same for control and
		Conditions		exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.

Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation

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Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 792357 Table: 1 of 4

Media, Path:

Taxa, Species, Age: Vegetation; Vascular Plants; *Nicotinana tobacum*; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 792357

Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited
Domain 6: Confounding	ng / Variable Coi	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Preser	ntation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: Germination

Overall Quality Determination

Low

HERO ID: 792357 Table: 2 of 4

Stud	y Citation:	Jia, Z. H., Yi, J. H., Su,	Y. R., Shen, H. (201	1). Autotoxic substances in the roo	ot exudates from continuous tobacco	o cropping. Allelopathy Journal 27(1):87-
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96. Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days **Duration:**

Exposure Route,

Media, Path:

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vegetation; Vascular Plants; Nicotinana tobacum; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Reproductive/Teratogenic

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID.	172331			
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Zomani J. Exposure Cir	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (6.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.
Domain 4: Test Organisi	m			
Č	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported
			tinued on nex	

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 792357 Table: 2 of 4

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Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Terrestria

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vegetation; Vascular Plants; *Nicotinana tobacum*; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 792357

Additional Comments:

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited
Domain 6: Confound	ling / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental
		Design and Procedures		conditions
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Pres	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Overall Quality Determination

Germination.

Low

HERO ID: 792357 Table: 3 of 4

Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age:

Vegetation; Vascular Plants; Nicotinana tobacum; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID.	172331			
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes
	Metric 6:	Randomized Allocation	Low	Researchers did not report how seeds were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Zomani J. Exposure Cir	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (6.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.
Domain 4: Test Organisi	m			
Č	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.
Domain 5: Outcome Ass	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported
			tinued on nex	

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 792357 Table: 3 of 4

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Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Terrestrial; Water; N

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vegetation; Vascular Plants; Nicotinana tobacum; Hong da; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Additional Comments:

Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 792357

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited
Domain 6: Confound	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental
		Design and Procedures		conditions
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Overall Quality Determination

Low

Growth (Development-Slowed, Retarded, Delayed or Non-development, Response Site: Not reported)

HERO ID: 792357 Table: 4 of 4

Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Vegetation; Vascular Plants; *Nicotinana tobacum*; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported

Taxa, Species, Age: Health Outcome:

Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID.	172331			
Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	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 Cl	haracterization			
Boniani 3. Exposure C.	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The composition of the containers the filter papers were kept in during the experiment was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	Low	Exposure concentrations were above the solubility limit (6.2 mg/L at 25C). The lowest concentration, 0.1 mM, corresponds to approximately 27.8 mg/L.
Domain 4: Test Organis	sm			
	Metric 13:	Test Organism Characteristics	Low	The source of the test seeds was not clear.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms or replicates was not reported.
Domain 5: Outcome As	ssessment			
	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.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 792357 Table: 4 of 4

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Study Citation: Jia, Z. H., Yi, J. H., Su, Y. R., Shen, H. (2011). Autotoxic substances in the root exudates from continuous tobacco cropping. Allelopathy Journal 27(1):87-

96.

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Terrestrial; Water; No.

Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vegetation; Vascular Plants; *Nicotinana tobacum*; G168; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 792357

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Analy	veie		
Domain 7. Data 1105	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 growth (Development-Slowed, Retarded, Delayed or Non-development, Response Site: Not reported).

Overall Quality Determination

Low

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
	: F : (1T : 1 101 : (14/0) 15/0 1574

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Cyprinodon variegatus; Juvenile

Health Outcome: M

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature reported without CASRN
	Metric 2:	Test Substance Source	Low	Reported as provided by manufacturer from commercially available batches. Manufacture name and batch number not provided. No analytical data reported.
	Metric 3:	Test Substance Purity	High	At least 95% purity
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Negative control reported
	Metric 5:	Negative Control Response	High	Control response acceptable
	Metric 6:	Randomized Allocation	Low	Allocation method not reported.
Domain 3: Exposure Cl	haracterization			
, , , , , , , , , , , , , , , , , , ,	Metric 7:	Experimental System/Test Media Preparation	Medium	Experimental system well described. However, headspace or measures to prevent volatilization not reported.
	Metric 8:	Consistency of Exposure	High	Exposure administration consistent across groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at start and end of test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure appropriate for test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure levels appropriate. Range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	Test performed at or below water solubility
Domain 4: Test Organis	sm			
zomum w rest organi.	Metric 13:	Test Organism Characteristics	Low	Source not reported.
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for test reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome As	ssessment			
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions appropriate for test.
		Cont	tinued on nex	at page

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Cyprinodon variegatus*; Juvenile

Health Outcome: M Chemical: D

Mortality

Diisobutyl Phthalate- Parent compound

HERO ID: 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.
	Metric 18:	Consistency of Outcome	High	Outcome assessment consistent across groups.
		Assessment		
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences between groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination

phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Danio rerio; AB Strain; Embryo

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 2298079

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	The CAS numbers and structures for BBP, DBP, DEHP, DIDP, and DINP are reported.
	Metric 2:	Test Substance Source	High	Sources were listed.
	Metric 3:	Test Substance Purity	Low	Purity/grade were not reported.
Domain 2: Test Design				
	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 C	haracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	Dilution of the test substance into medium was not well described (unclear if embryo rearing medium was utilized) and the test substance was not renewed over 72 hr.
	Metric 8:	Consistency of Exposure	Medium	Exposures appear to have been administered consistently.
	Metric 9:	Administration Measurement of Test Substance	Low	Concentrations are reported as nominal.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	Acute exposures were 72-hr for embryos, which is slightly shorter than the standard 96-hour acute bioassay.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors reported conducting exposures at 9 concentrations: 500, 100, 50, 10, 1.5, 0.6, 0.3, 0.06, 0.01 mg/L.
	Metric 12:	Testing at or Below Solubility Limit	Low	Several concentrations were above estimated solubility limits, including 3-4 treatment concentrations (BBP, DBP) or 7 concentrations (DEHP, DIDP, and DINP). Methanol was utilized to improve solubility.
Domain 4: Test Organis	sm			
31g	Metric 13:	Test Organism Characteristics	High	Source and strain were reported and husbandry methods were adequately described.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation was not reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each concentration was represented by 20 embryos (one embryo per well).

Domain 5: Outcome Assessment

Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2298079 Table: 1 of 1

... continued from previous page

Study Citation: Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of

phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Danio rerio*; AB Strain; Embryo

Health Outcome: Chemical: Mortality

Diisobutyl Phthalate- Parent compound

HERO ID: 2298079

Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	The 24 well plates were described but there lacked details on water conditions (or use of embryo rearing medium) and temperature.
	Metric 17:	Outcome Assessment Methodology	Low	It was not reported how mortality was determined in embryos.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and control groups at 72 hr post treatment.
Domain 6: Confound	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate that animal health or attrition interfered with the bioassay.
Domain 7: Data Prese	entation and Anal	lysis		
	Metric 21:	Statistical Methods	Low	There were no details on the LC50 estimation methods.
	Metric 22:	Reporting of Data	Low	The authors report LC50 values for BBP and DBP (and LC50 not attained for DEHP, DINP, and DIDP). Mortality was not reported for each treatment group or for the controls.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	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
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organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Lepomis macrochirus; Juvenile

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substa	ince			
	Metric 1:	Test Substance Identity	Low	The test substance was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported as provided by a manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.
Domain 2: Test Design	1			
Domain 2. Test Design	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The negative control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure C	`haracterization			
Zomani U. Ziipoowi U	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure were appropriate for this test.
	Metric 11:	Number of Exposure Groups/ 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 Organi	ism			
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation for this test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.

Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Lepomis macrochirus*; Juvenile

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 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	High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confoundir	ng / Variable Con	ntrol		
	Metric 19: Confounding		High	Environmental conditions were consistent across groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Preser	ntation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

Overall Quality Determination

Study Citation:	Bionomics., EG&G (1983). Exhibit III: A	cute toxicity of thirteen phthalate este	rs to bluegill (Lepomis macrochirus).

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mortalit

Chemical: Diisobutyl Phthalate- Parent compound

1310201			
	Metric	Rating	Comments
ice			
Metric 1:	Test Substance Identity	High	The test substance- Dibutyl phthalate (DBP) was identified by chemical name and CASRN (84-74-2).
Metric 2:	Test Substance Source	Low	The source was not reported and the test substance identity was not analytically verified by the performing labortory.
Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported.
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.
naracterization			
Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/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 study authors and adequate to address the purpose of the study. For Dibutyl phthalate (DBP), a preliminary test was conducted, which indicated that it was not toxic below the water solubility limit. A corroborative test was then conducted exposing the bluegill to a single replicated concentration of Dibutyl phthalate (DBP) representing its limit of water solubility.
Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at the water solubility limit.
·m			
	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
		C	The test organisms were acclimatized to test conditions and all pretreatment conditions
THOUSE IT.	Conditions	111611	were the same for control and exposed organisms.
	Cont	tinued on nex	t nage
	Metric 1: Metric 2: Metric 3: Metric 4: Metric 5: Metric 6: maracterization Metric 7: Metric 8: Metric 9: Metric 10: Metric 11:	Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Purity Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit Sm Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Conditions	Metric 1: Test Substance Identity High Metric 2: Test Substance Source Low Metric 3: Test Substance Purity Low Metric 4: Negative Controls High Metric 5: Negative Control Response High Metric 6: Randomized Allocation Medium Maracterization Metric 7: Experimental System/Test Media Low Preparation Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency High Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit High Metric 13: Test Organism Characteristics High Metric 14: Acclimatization and Pretreatment High

Diisobutyl Phthalate HERO ID: 1316201 Table: 1 of 1

		conti	nued from p	revious page				
Study Citation:				nthalate esters to bluegill (Lepomis macrochirus).				
Ouration:		ation: 0 - 4 days (0-96h); Exposure Duratio						
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:								
Гаха, Species, Age:	Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported							
Health Outcome:	Mortality							
Chemical:	•	hthalate- Parent compound						
HERO ID:	1316201							
Domain		Metric	Rating	Comments				
	Metric 15:	Number of Organisms and	Medium	There were ten bluegill in each test jar, and they were tested in duplicates.				
		Replicates per Group						
D : 5 O . A								
Domain 5: Outcome As		A do	TT: _L					
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were conducive to maintenance of health. The biomass loading was appropriate.				
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of				
	Wietire 17.	Succession reseasement rectioned oragi	111511	interest.				
	Metric 18:	Consistency of Outcome	High	Details of the outcome assessment protocol were reported, and outcomes were assessed				
		Assessment		consistently across study groups.				
D : (C (1	/W : 11 G	. 1						
Domain 6: Confounding			TT' 1					
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.				
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
	Metric 20.	Outcomes Officiated to Exposure	Medium	There was no information in the study on outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	vsis						
	Metric 21:	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:				e test (following negative findings from a preliminary test at concentrations below le replicated concentration of Dibutyl phthalate (DBP) representing it's limit of w				

Overall Quality Determination

Study Citation:	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bulletin of Environmental
	Contamination and Toxicology 26(4):446-452.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 18064; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983

Domain		Metric	Rating	Comments
Domain 1: Test Subst	tance			
	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 Desig	gn			
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure	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 spacing of exposure levels, but cited methods suggest using a minimum of five treatment levels.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The authors reported this as "precipitate." However, the highest reported LC50 value is 2.1 mg/L, compared to the solubility reported in the DBP Final Scope of 11.2 mg/L at 25C.
Domain 4: Test Organ	nism			
Tool Olgan	Metric 13:	Test Organism Characteristics	Medium	There are minor uncertainties about the source and characteristics of test organisms because the authors use a generalized description for all reported tests.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms for the 48 hours prior to testing.
		Conti	nued on next pa	nge

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 18064 Table: 1 of 1

... continued from previous page

Study Citation: Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (Lepomis macrochirus). Bulletin of Environmental

Contamination and Toxicology 26(4):446-452.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Lepomis macrochirus; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Mortality

lealth Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 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 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: Confound	ling / Variable Co	ntrol		
Domain of Comount	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 Pres	sentation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described (method of moving average angles or Wilcoxon log probit).
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the tables.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Occurrence of unexpected outcomes was not addressed.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation: EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions. **Duration:**

Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route,**

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Oncorhynchus mykiss; Juvenile

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 6571362

Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be the Chemical Manufacturers Association in Washington DC, and was analytically verified by GC-MS.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%.
Domain 2: Test Design	1			
C	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.
	Metric 5:	Negative Control Response	Low	The negative control for the preliminary test was not reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how juvenile fish were allocated into study groups in the preliminary test.
Domain 3: Exposure C	Characterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test concentrations.
	Metric 8:	Consistency of Exposure Administration	Low	All exposures for the preliminary test were conducted for 13 days. The test chambers for this portion of the study were not described. Little other information was provided regarding test conditions for the preliminary study.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if test concentrations were measured in the preliminary study.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 13 days. This was adequate for a preliminary test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups as well as a negative control and a solvent control. Thi is typical for testing, and spacing was appropriate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.
Domain 4: Test Organ	sm			
8	Metric 13:	Test Organism Characteristics	High	The trout were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation was not reported for the preliminary test.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The number of fish per replicate and the number of replicates were not reported for the preliminary study.

Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 6571362 Table: 1 of 1

... continued from previous page

Study Citation: EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Vertebrate Health Outcome: Mortality

pecies, Age: Vertebrate; Fish; *Oncorhynchus mykiss*; Juvenile

Chemical: Diisobutyl Phthalate- Parent compound HERO ID: 6571362

HERO ID:	03/1302			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Test conditions were not reported for the preliminary study.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest–fish survival in the terms of LC50 values for 96h and 13d.
	Metric 18:	Consistency of Outcome Assessment	Low	Minimal details were provided regarding outcome assessment for the preliminary study.
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Pres	sentation and Anal	lysis		
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately for the preliminary study.
	Metric 22:	Reporting of Data	Low	Data were only reported in the text for the preliminary test.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported for the preliminary study.

Overall Quality Determination

Additional Comments:

Medium

preliminary test protocol and procedures was reported. Mortality was the outcome of interest.

This evaluation is for the preliminary test conducted with juvenile rainbow trout. 96h and 13d LC50 values were reported. Little information regarding

Study	Citation:
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EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

HERO ID: 6571362 Table: 1 of 2

Duration:

Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age:

Vertebrate; Fish; Oncorhynchus mykiss; Embryo

Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID:

6571362

Domain		Metric	Rating	Comments
Domain 1: Test Substan	nce			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	Low	The source of the DBP was reported to be the Chemical Manufacturers Association in Washington DC. It was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response and the vehicle control response were reported in Tables 4, 5, and in B3-B7.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly distributed to each exposure group.
Domain 2: Evnagura C	haraatarization			
Domain 3: Exposure C	Metric 7:	Experimental System/Test Media	Low	Little information was provided on the preparation of the test concentrations.
	metric 7.	Preparation	2011	Entire information was provided on the preparation of the less concentrations.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures were for 99 days unless all the organisms in the test concentration had died prior to the end of the study. All the tests were conducted in 14 x 53 x 25cm with 15L of test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	The test concentrations were measured by two methods, a radiochemical analytical method and via GC/MS.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 99 days. This was adequate to observe a response across study groups.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups as well as a negative control and a solvent control. This is typical for testing, and spacing was appropriate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.
Domain 4: Test Organia	sm			
1000 01guin	Metric 13:	Test Organism Characteristics	High	The embryos and sperm were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.
	Metric 14:	Acclimatization and Pretreatment	Medium	Acclimation was reported, but the duration was not reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	There were 30 embryos per test chamber with two replicates. Two replicates is less than is typical, thus the low ranking.

Continued on next page ...

Diisobutyl Phthalate **Environmental Hazard Evaluation** HERO ID: 6571362 Table: 1 of 2

... continued from previous page

Study Citation: Duration:

EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Vertebrate; Fish; Oncorhynchus mykiss; Embryo

Taxa, Species, Age: **Health Outcome:**

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 6571362

sment Methodology Outcome	High High High	Embryos were held at 10C for the first 6 weeks and then the temperature was gradually increased to 12.5C over week 7 to the end of the study. Embryos were kept in the dark until day 43, at the start of swim-up, and then kept at a photoperiod of 14L:10D. Fry were fed starter mash and live brine shrimp. The outcome assessment methodology addressed or reported the intended outcome of interest–lengths and weights of larval fish, as well as any other sublethal effects. Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Caliper lengths and photographic lengths were taken at the end of the study, and wet weights and dry weights were both taken at the end of the study. There were no reported differences among the study groups in environmental conditions
sment Methodology Outcome ariables in Test	High High	increased to 12.5C over week 7 to the end of the study. Embryos were kept in the dark until day 43, at the start of swim-up, and then kept at a photoperiod of 14L:10D. Fry were fed starter mash and live brine shrimp. The outcome assessment methodology addressed or reported the intended outcome of interest–lengths and weights of larval fish, as well as any other sublethal effects. Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Caliper lengths and photographic lengths were taken at the end of the study, and wet weights and dry weights were both taken at the end of the study.
Outcome ariables in Test	High	interest-lengths and weights of larval fish, as well as any other sublethal effects. Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Caliper lengths and photographic lengths were taken at the end of the study, and wet weights and dry weights were both taken at the end of the study.
ariables in Test		consistently across study groups. Caliper lengths and photographic lengths were taken at the end of the study, and wet weights and dry weights were both taken at the end of the study.
	High	There were no reported differences among the study groups in environmental conditions
	High	There were no reported differences among the study groups in environmental conditions
		or other factors that could influence the outcome assessment.
	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
ods	High	Statistical methods were described in the "statistical analysis" section of the report.
	High	Body weights and lengths were provided as well as other sublethal effects were reported in Tables 4, 5, and B3-B7.
Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the tables.
1	ita	Unexpected Outcomes High

Overall Quality Determination

Study Citation:

EnviroSystem, (1991). Early life-stage toxicity of di-n-butyl phthalate (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through conditions.

Duration:

Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Oncorhynchus mykiss; Embryo

Health Outcome:

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 6571362

Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	High	The source of the DBP was reported to be the Chemical Manufacturers Association in Washington DC, and was analytically verified by GC-MS.
	Metric 3:	Test Substance Purity	High	The purity of the DBP was reported to be 99.9%.
Domain 2: Test Design	l			
3	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control and a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response and the vehicle control response were reported in Table 3 and in Tables B1 and B2.
	Metric 6:	Randomized Allocation	Medium	Embryos were randomly distributed to each exposure group.
Domain 2: Evnogura C	haraatarization			
Domain 3: Exposure C	Metric 7:	Experimental System/Test Media	Low	Little information was provided on the preparation of the test concentrations
	Menic /:	-	Low	Little information was provided on the preparation of the test concentrations.
	Metric 8:	Preparation Consistency of Exposure	Medium	All exposures were for 99 days unless all the organisms in the test concentration had
	Wedle 6.	Administration	Wediam	died prior to the end of the study. All the tests were conducted in 14 x 53 x 25cm with 15L of test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	The test concentrations were measured by two methods, a radiochemical analytical method and via GC/MS.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 99 days. This was adequate to observe a response across study groups.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups as well as a negative control and a solvent control. This is typical for testing, and spacing was appropriate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit, and a vehicle solvent was used. The solvent control had an appropriate response.
D : 4 T + 0				
Domain 4: Test Organi		T. (O.). Cl. ().	TT' 1	
	Metric 13:	Test Organism Characteristics	High	The embryos and sperm were from Mt. Laassen Trout Farms and were the Hildebrand strain. They were certified to be disease free.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation was reported, but the duration was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 30 embryos per test chamber with two replicates. Two replicates is less than is typical, thus the low ranking.

Domain 5: Outcome Assessment

Continued on next page ...

Diisobutyl Phthalate HERO ID: 6571362 Table: 2 of 2

		conti	nued from p	revious page
Study Citation: Duration: Exposure Route, Media, Path:	Overall Dura	ation: > 21 days; Exposure Duration: > 21	days	te (DnBP) to the rainbow trout (Oncorhynchus mykiss) under flow-through condition chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; F	Fish; Oncorhynchus mykiss; Embryo		
Health Outcome:	Mortality			
Chemical:	Diisobutyl P	hthalate- Parent compound		
HERO ID:	6571362			
Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Embryos were held at 10C for the first six weeks and then the temperature was gradually increased to 12.5C over week seven to the end of the study. Embryos were kept in the dark until day 43, at the start of swim-up, and then kept at a photoperiod of 14L:10D. Fry were fed starter mash and live brine shrimp.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest-embryo/larval survival and percent hatch.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Embryos and larvae were observed daily for mortalities.
Domain 6: Confounding	/ Variable Co	ntrol		
zomum or comounum	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Present	ation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "statistical analysis" section of the report.
	Metric 22:	Reporting of Data	High	Mortality data for the control responses and the exposure responses were reported in Tables 3, B1, and B2 and were adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.
Additional Comments:	•	of the evaluation was on the effect of D as selected as the outcome of interest.	BP on embr	yo survival, percent hatch, and larval survival at various points in the 99 day study

Overall Quality Determination

Study Citation:	Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report)
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report no BW-83-3-1373.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Species, Age: Vertebrate; Fish; Oncorhynchus mykiss (Salmo gairdneri); Adult

Health Outcome: Mortality

Chemical: Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.
	Metric 2:	Test Substance Source	High	The test substance was obtained from General Electric Company, Hudson Falls, NY on 11 and 18 December 1981.
	Metric 3:	Test Substance Purity	High	The test substance was reported as "100% active ingredient." Though absolute purity is doubtful, this can be interpreted as an indication of very high purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were included.
	Metric 5:	Negative Control Response	High	There was no unacceptable mortality in controls.
	Metric 6:	Randomized Allocation	Medium	Trout were randomly distributed among aquaria.
Domain 3: Exposure C	haracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	A flow-through system with daily replenishment of solution was used. A detailed diluter design for mixing phthalates was described in Appendix I.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across substance groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Test concentrations were measured during the experiment. Endpoints were based on measured concentrations.
	Metric 10:	Exposure Duration and Frequency	High	This was a 96-h acute exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The range of concentrations and number of groups were acceptable to determine LC50 values.
	Metric 12:	Testing at or Below Solubility Limit	Medium	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 Organi	sm			
C	Metric 13:	Test Organism Characteristics	High	Fish were obtained from commercial suppliers in Maryland and Montana. Lot numbers were given.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatization was for a minimum of 14 days in holding tanks.
		Cont	tinued on nex	at page

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5530771 Table: 1 of 1

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Study Citation: Bionomics,, EG&G (1983). Acute toxicity of fourteen phthalate esters to rainbow trout (Salmo gairdneri) under flow-through conditions (final report)

report no BW-83-3-1373.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Oncorhynchus mykiss (Salmo gairdneri); Adult

Health Outcome:

Mortality

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 5530771

Domain		Metric	Rating	Comments
	Metric 15:	Number of Organisms and	Medium	There were ten fish per group and two replicates per concentration.
		Replicates per Group		
Domain 5: Outcome Asse	essment			
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for husbandry of rainbow trout.
	Metric 17:	Outcome Assessment Methodology	High	The outcome was assessed appropriately (mortality).
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently among study groups.
Domain 6: Confounding			Hich	
	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 Presentat	tion and Anal	ysis		
	Metric 21:	Statistical Methods	Low	The LC50 was calculated by a customized computer program using moving average angle analysis, probit analysis, or binomial probability. Details of the program were not reported.
	Metric 22:	Reporting of Data	High	Data were reported for all groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Overall Quality Determination

None

Additional Comments:

Study	Citation:	EAG Laboratories, (2018)	Dibutyl phthalate:	Medaka extended one	generation repr	roduction test (f	inal report).
~~~~.		2110 240014101105, (2010)	Diodelji piididiate.	Tite dania citteriaca cite	Serrer acron rep.		mar reports.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Oryzias latipes*; Adult

**Health Outcome:** Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID:	10004180			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	ince			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Design	1			
C	Metric 4:	Negative Controls	High	Study authors used a concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	Characterization			
•	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations
	Metric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on results.
	Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dos
		Spacing of Exposure Levels		response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit
Domain 4: Test Organi	ism			
	Metric 13:	Test Organism Characteristics	Low	The test organisms were not adequately described and assumed to be same source as definitive test
	Metric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized
	Metric 15:	Conditions 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 A		A da	TT: _1.	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	Low	Environmental conditions were not sufficiently reported to evaluate if adequate
		Conti	nued on next pa	nge

Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Aquatic (freshwater **Media, Path:** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 1 of 2

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were not
		Assessment		reported
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental
		Design and Procedures		conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Sufficient data were provided to conduct an independent statistical analysis.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: range finder

# **Overall Quality Determination**

# Medium

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** 

Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** 

ne: Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
M	etric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.
M	etric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
M	etric 3:	Test Substance Purity	High	Chemical purity was reported as 99.3%.
Domain 2: Test Design				
	etric 4:	Negative Controls	High	Study authors used a concurrent negative control group.
	etric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.
	etric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Charac	terization			
•	etric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
M	etric 8:	Consistency of Exposure	Medium	Reporting omissions are unlikely to have a substantial impact on the results.
M	etric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not measured or measurements were not reported.
M	etric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
M	etric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels	C	response.
M	etric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism				
	etric 13:	Test Organism Characteristics	Low	The test organisms were not adequately described, and they were assumed to be from the same source as the definitive test.
M	etric 14:	Acclimatization and Pretreatment	Low	The study did not report whether test organisms were acclimatized.
M	etric 15:	Conditions 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 Assessi		A.I. STD v.G. WY	*** *	
	etric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
M	etric 17:	Outcome Assessment Methodology	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
M	etric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.

### Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 10064186 Table: 2 of 2

### ... continued from previous page

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Oryzias latipes*; Adult

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Present	Metric 21:	ysis 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.

# **Overall Quality Determination**

# Medium

Study Citation:	EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).
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**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Aquatic (freshwater); Water; Not determined by study author

Media, Path:

Chemical:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Repro

Reproductive/Teratogenic
Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce						
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#			
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.			
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable			
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups			
D : 1 E G							
Domain 3: Exposure Ch			TT: 1				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail			
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups			
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals			
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose			
	wietiie 11.	Spacing of Exposure Levels	mgn	response			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit			
Domain 4: Test Organis	m						
Domain 4. Test Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source			
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions			
	metric 1 i.	Conditions	111511	The test organisms were decimalized to test conditions			
	Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-			
		Replicates per Group		ize toxicological effects			
Domain 5: Outcome As	sessment						
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health			
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest			
	Continued on next page						

Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 1 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups
		Assessment		
Domain 6: Confound	ding / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Pres	sentation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained

Additional Comments: generational effects

# **Overall Quality Determination**

HERO ID: 10064186 Table: 2 of 8

**Study Citation:** 

EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** 

Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** 

Skin & Connective Tissue

10064186

Chemical:

Diisobutyl Phthalate- Parent compound

Assessment

HERO ID:

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
M	etric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#
M	etric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
M	etric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Design				
	etric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
M	etric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
M	etric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups
Domain 3: Exposure Charac	eterization			
•	etric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
M	etric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
M	etric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
M	etric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type
M	etric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels	C	response
M	etric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit
Domain 4: Test Organism				
	etric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
M	etric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions
M	etric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-
		Replicates per Group		ize toxicological effects
Domain 5: Outcome Assessi	ment			
M	etric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
M	etric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest (page 37)
M	etric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups

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Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 2 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Skin & Connective Tissue

Chemical: Diisobutyl Phthalate- Parent compound

Domain		Metric	Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7. Data Present	ation and Anal	ia		
Domain 7: Data Present				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained
Additional Comments:	nts: This is for the anal fin papillae evaluation.			
O!! O!'4 D-4			TT! -1-	
Overall Quality Determination			High	

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Oryzias latipes*; Adult

**Health Outcome:** Hepatic/Liver

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

HERO ID:	10004180			
Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	The chemical was identified by name and CAS#.
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.3%.
Domain 2: Test Design	l			
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable.
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups.
Domain 3: Exposure C	haracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels	J	response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organi	sm			
Domain II Test organi	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions.
	- · · ·	Conditions	8	<u> </u>
	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 A	cceccment			
Domain J. Outcome A	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism
			C	health.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest (pages 37-39).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups

Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 3 of 8

Taxa, Species, Age:

Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Hepatic/Liver

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
D		:		
Domain 7: Data Present		•		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
				(Appendix 8).
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.
Additional Comments:	This is for th	e liver histopathology evaluation.		

## **Overall Quality Determination**

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** 

Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Mortality

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups
				, , , , , , , , , , , , , , , , , , , ,
Domain 3: Exposure Ch	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	wieure 11.	Spacing of Exposure Levels	Iligii	response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit
			-	
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects
Domain 5: Outcome As				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

Diisobutyl Phthalate **Environmental Hazard Evaluation** 

### ... continued from previous page

EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report). **Study Citation:** 

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 4 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Mortality

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confoundi	ng / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
Domain 7: Data Prese	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Prese		Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Prese	ntation and Anal Metric 21:	Outcomes Unrelated to Exposure  ysis Statistical Methods	High	Statistical methods were adequately described
Domain 7: Data Prese	ntation and Anal	Outcomes Unrelated to Exposure ysis		

### **Overall Quality Determination**

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age:Vertebrate; Fish; Oryzias latipes; AdultHealth Outcome:Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

HERO ID:	10064186			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	ance			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Desig	n			
_	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups
Domain 3: Exposure (	Characterization			
r i	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels		response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit
Domain 4: Test Organ	nism			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects
Domain 5: Outcome A	\ccasemant			
Domain 3. Outcome P	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups
		Assessment	8	g.oup

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Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 5 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variab	· Control		
Metric	9: Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
	Design and Procedures		
	2 coign and 110 codares		
Metric	0: Outcomes Unrelated to Exposure	High	there were no differences among groups
	0: Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Presentation and Metric	On Outcomes Unrelated to Exposure  Analysis  Statistical Methods	High	there were no differences among groups  Statistical methods were adequately described
Domain 7: Data Presentation and	On Outcomes Unrelated to Exposure  Analysis  Statistical Methods		

## **Overall Quality Determination**

Study Citation: Duration: Exposure Route, Media, Path:	Overall Dura	ation: $> 21$ days; Exposure Duration: $> 2$	1 days	e generation reproduction test (final report).  chemical of interest in exposure water, but unable to determine exact uptake route)	
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; Fish; <i>Oryzias latipes</i> ; Adult Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Endocrine toxicity-Reproductive/Teratogenic Diisobutyl Phthalate- Parent compound 10064186				
Domain		Metric	Rating	Comments	
Domain 1: Test Substan	<u> </u>				
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#	
	Metric 2: Metric 3:	Test Substance Source Test Substance Purity	High High	The test substance identity was analytically verified by the performing laboratory. Chemical purity reported as 99.3%	
	Wietite 3.	rest substance runty	mgn	Chemical purity reported as 97.3 to	
Domain 2: Test Design					
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group	
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable	
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups	
Domain 3: Exposure Ch	aracterization				
z omam et znpesare en	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail	
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups	
	Metric 9:	Administration Measurement of Test Substance	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals	
	Metric 10:	Concentration Exposure Duration and Frequency	Uiah	·	
	Metric 10:	Number of Exposure Groups/	High High	The duration of exposure was suitable for the study type  The number of exposure groups and spacing of exposure levels were suitable for a dose	
	MEUIC II:	Spacing of Exposure Levels	High	response	
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit	
Domain 4: Test Organis	m				
Domain 4. 10st Organis	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source	
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions	
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-	
		Replicates per Group		ize toxicological effects	
Domain 5: Outcome As	sessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism	

### Continued on next page ...

High

High

Outcome Assessment Methodology

Consistency of Outcome

Assessment

Metric 17:

Metric 18:

health

The outcome assessment methodology reported the intended outcome of interest

outcomes were assessed consistently across study groups

Diisobutyl Phthalate **Environmental Hazard Evaluation** 

### ... continued from previous page

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 6 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function-Endocrine toxicity-Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confoundi	ng / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Prese		5		
	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:	<b>Explanation of Unexpected Outcomes</b>	High	unexpected outcomes were satisfactorily explained
Additional Comments	generational	effects		

# **Overall Quality Determination**

HERO ID: 10064186 Table: 7 of 8

**Study Citation:** 

EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** 

Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Vertebrate; Fish; Oryzias latipes; Adult Taxa, Species, Age:

**Health Outcome:** 

Endocrine Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric	1: Test Substance Identity	High	Chemical was identified by name and CAS#
Metric	2: Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
Metric	3: Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Design			
Metric	4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
Metric	9	High	The biological response of the negative control group was reported and reasonable
Metric		Medium	The study reported that organisms were randomly allocated into study groups
Domain 3: Exposure Characteriz	ration		
Metric		High	The experimental system and methods for preparation of test media were described in adequate detail
Metric	, i	High	exposures were administered consistently across study groups
Metric	9: Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
Metric		High	The duration of exposure was suitable for the study type
Metric		High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	Spacing of Exposure Levels	C	response
Metric		High	Exposure concentrations were below the water solubility limit
Domain 4: Tost Organism			
Domain 4: Test Organism  Metric	13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
Metric	E	High	The test organisms were acclimatized to test conditions
Wetric	Conditions	nigii	The test organisms were accumulatized to test conditions
Metric		Medium	The numbers of test organisms and replicates were reported and sufficient to character-
	Replicates per Group		ize toxicological effects
Domain 5: Outcome Assessment			
		III al-	
Metric	16: Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
Metric	17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest (pages 37-39)
Metric	18: Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

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Diisobutyl Phthalate Environmental Hazard Evaluation

### ... continued from previous page

Study Citation: EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 7 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** Endocrine

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions
	Metric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Presen	Metric 21: Metric 22: Metric 23:	ysis Statistical Methods Reporting of Data Explanation of Unexpected Outcomes	High High High	Statistical methods were adequately described  Data for exposure-related findings were presented for each treatment and control group (Appendix 8)  unexpected outcomes were satisfactorily explained

## **Overall Quality Determination**

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:
Taxa, Species, Age: Vertebrate; Fish; *Oryzias latipes*; Adult

**Health Outcome:** Renal/Kidney

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 10064186

HERU ID:	10004180			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	High	Chemical was identified by name and CAS#
	Metric 2:	Test Substance Source	High	The test substance identity was analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.3%
Domain 2: Test Design	1			
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable
	Metric 6:	Randomized Allocation	Medium	The study reported that organisms were randomly allocated into study groups
Domain 3: Exposure C	haracterization			
r	Metric 7:	Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail
	Metric 8:	Consistency of Exposure	High	exposures were administered consistently across study groups
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using appropriate analytical technologies and methods, however measured concentrations were consistently lower than nominals
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels	8	response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit
Domain 4: Test Organi	ism			
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source
	Metric 14:	Acclimatization and Pretreatment	High	The test organisms were acclimatized to test conditions
	Metric 15:	Conditions Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to character-
		Replicates per Group		ize toxicological effects
Domain 5: Outcome A				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest (pages 37-39)
	Metric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups

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Diisobutyl Phthalate **Environmental Hazard Evaluation** 

### ... continued from previous page

**Study Citation:** EAG Laboratories, (2018). Dibutyl phthalate: Medaka extended one generation reproduction test (final report).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 10064186 Table: 8 of 8

Taxa, Species, Age: Vertebrate; Fish; Oryzias latipes; Adult

**Health Outcome:** 

**Overall Quality Determination** 

Renal/Kidney

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 10064186

Domain		Metric	Rating	Comments
Domain 6: Confounding / V	ariable Con	trol		
M	Ietric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
M	Ietric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Presentation	n and Anals	veje		
	letric 21:	Statistical Methods	High	Statistical methods were adequately described
			_	1 ,
M	Ietric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
		F 1 4 6H	*** 1	(Appendix 8)
M	letric 23:	Explanation of Unexpected Outcomes	High	unexpected outcomes were satisfactorily explained
Additional Comments: Tl	his is for the	e kidney histopathology evaluation.		

Study Citation:	Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of
	phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Vertebrate; Fish; Oryzias melastigma; ChgH-EGFP; Larvae
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic
Chemical:	Diisobutyl Phthalate- Parent compound
HERO ID:	2298079

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	The CAS numbers and structures for BBP, DBP, DEHP, DIDP, and DINP were reported.
	Metric 2:	Test Substance Source	High	The sources were reported.
	Metric 3:	Test Substance Purity	Low	Purity/grade were not reported.
Domain 2: Test Design				
8	Metric 4:	Negative Controls	High	Both a blank control and solvent control were used in the acute bioassays.
	Metric 5:	Negative Control Response	High	Control responses (blank and solvent) are shown in Figure 3. Positive control responses are shown in Figures 3, 4, and 5.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Ch	naracterization			
Domain S. Enposare C.	Metric 7:	Experimental System/Test Media	Low	Preparation of test substances and dilution into test medium was not well described.
		Preparation		
	Metric 8:	Consistency of Exposure	High	Exposures appear to have been administered consistently.
	Metric 9:	Administration Measurement of Test Substance	Low	Concentrations are reported as nominal.
	Metric 10:	Concentration Exposure Duration and Frequency	Low	Exposures were 24-hr for embryos, which is shorter than the typical 72-96 hrs utilized in other transgenic fish embryo studies.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Five concentrations were utilized in the pre-testing, from which one concentration per phthalate was utilized in the formal testing.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations utilized in the formal testing did not exceed solubility (BBP and DBP) and slightly exceeded solubility (DIDP, DINP, DEHP). Methanol was utilized to increase solubility.
Domain 1: Test Organis	·m			
Domain 4: Test Organis	Metric 13:	Test Organism Characteristics	Medium	The source for the transgenic medaka was cited as Chen et al 2007 and Cheng and Chen
	MEUIC 13.	Test Organism Characteristics	Mediuili	2013, but it was not well described in the cited sources.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of embryos in 24-well plates was not reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each concentration was tested in triplicate with eight embryos per replicate.

### Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 2298079 Table: 1 of 1

### ... continued from previous page

Study Citation: Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F., F.W., Xu, L., S.J., Ho, K. C. (2014). Toxicity and estrogenic endocrine disrupting activity of

phthalates and their mixtures. International Journal of Environmental Research and Public Health 11(3):3156-3168.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Oryzias melastigma*; ChgH-EGFP; Larvae

Health Outcome: Chemical:

Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity-Endocrine toxicity-Reproductive/Teratogenic

**Chemical:** Diisobutyl Phthalate- Parent compound

**HERO ID:** 2298079

Domain	Metric		Rating	Comments	
Domain 5: Outcome Assessi	ment				
M	letric 16:	Adequacy of Test Conditions	Low	The 24 well plates were described but no other details on environmental conditions were provided.	
M	letric 17:	Outcome Assessment Methodology	Medium	Measurement of GFP in liver of embryos was described in the methods. Anesthesia of embryos prior to imaging was not described.	
M	letric 18:	Consistency of Outcome Assessment	High	The outcome assessment appeared to be consistently conducted across treatment and control groups at 72 hr exposure.	
Domain 6: Confounding / Va	ariable Con	trol			
M	letric 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.	
M	letric 20:	Outcomes Unrelated to Exposure	Medium	Nothing was reported to indicate that animal health or attrition interfered with the bioassay.	
Domain 7: Data Presentation	n and Analy	vsis			
M	letric 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.	
M	fetric 22:	Reporting of Data	Medium	Solvent control and positive control data were shown in Figure 3. Response for BBP treatment was shown in Figure 4 and co-treatment response for DBP, DEHP, DIDP, and DINP are shown in Figure 5.	
M	letric 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

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
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organisms. Environmental Toxicology and Chemistry 14(9):1569-1574. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Duration:** 

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Juvenile

**Health Outcome:** Mortality

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

	Metric	Rating	Comments
ice			
Metric 1:	Test Substance Identity	Low	Test substance nomenclature reported without CASRN
Metric 2:	Test Substance Source	Low	Reported as provided by manufacturer from commercially available batches. Manufacture name and batch number not provided. No analytical data reported.
Metric 3:	Test Substance Purity	High	At least 95% purity
Metric 4:	Negative Controls	High	Negative control reported
Metric 5:	Negative Control Response	High	Control response acceptable
Metric 6:	Randomized Allocation	Low	Allocation method not reported.
naracterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	Experimental system well described. However, headspace or measures to prevent volatilization not reported.
Metric 8:	Consistency of Exposure	High	Exposure administration consistent across groups.
Metric 9:	Administration Measurement of Test Substance	High	Sample extracts were analyzed by gas chromatography at start and end of test.
Metric 10:	Concentration Exposure Duration and Frequency	High	Duration and frequency of exposure appropriate for test.
		-	Exposure levels appropriate. Range finding test was performed.
		8	
Metric 12:	Testing at or Below Solubility Limit	High	Test performed at or below water solubility
m			
	Test Organism Characteristics	Low	Source not reported.
	Acclimatization and Pretreatment		Appropriate acclimation for test reported.
Metric 15:	Conditions Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test
	Replicates per Group		vessel.
sessment			
Metric 16:	Adequacy of Test Conditions	High	Environmental conditions appropriate for test.
Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.
	Cont	tinued on nex	at page
	Metric 2: Metric 3:  Metric 4: Metric 5: Metric 6:  Maracterization Metric 7: Metric 8: Metric 9: Metric 10: Metric 11: Metric 12:  Metric 12:  Metric 13: Metric 14: Metric 15:	Metric 1: Test Substance Identity Metric 2: Test Substance Source  Metric 3: Test Substance Purity  Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation  Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Number of Organisms and Replicates per Group  sessment Metric 16: Adequacy of Test Conditions Metric 17: Outcome Assessment Methodology	Metric 1: Test Substance Identity Low Metric 2: Test Substance Source Low  Metric 3: Test Substance Purity High  Metric 4: Negative Controls High Metric 5: Negative Control Response High Metric 6: Randomized Allocation Low  Maracterization Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure High Administration Metric 9: Measurement of Test Substance High Concentration Metric 10: Exposure Duration and Frequency High Metric 11: Number of Exposure Groups/ Metric 12: Testing at or Below Solubility Limit High  Metric 13: Test Organism Characteristics Low Metric 14: Acclimatization and Pretreatment High Conditions Metric 15: Number of Organisms and Medium Replicates per Group  sessment Metric 16: Adequacy of Test Conditions High

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 2

### ... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; *Pimephales promelas*; Juvenile

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments	
	Metric 18:	Consistency of Outcome	High	Outcome assessment consistent across groups.	
		Assessment			
Domain 6: Confound	ling / Variable Cor	ntrol			
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.	
		Design and Procedures			
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No reported differences between groups.	
Domain 7: Data Pres	entation and Anal	vsis			
	Metric 21:	Statistical Methods	High	Statistical methods performed and described.	
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints reported.	
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.	

Additional Comments: None

## **Overall Quality Determination**

HERO ID: 1321996 Table: 2 of 2

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vertebrate; Fish; Pimephales promelas; Juvenile

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

HERO ID.	1321990, LII	iked HERO ID(s). 1321990, 1310224		
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The source was provided by a manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Ch	aracterization			
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. Static tests were conducted in 19.6-L jars with 15 L of test solution. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure	High	Exposure administration was consistent across groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure levels were appropriate. A range finding test was performed.
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for the test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
		Cont	tinued on nex	xt page

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 2 of 2

### ... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Pimephales promelas*; Juvenile

**Health Outcome:** 

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 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	High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confound	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
		r		S
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

## **Overall Quality Determination**

Study Citation:	Mccarthy, J. F., Whitmore, D. K. (1985).	Chronic toxicity of di-n-butyl and di	i-n-octyl phthalate to daphnia-mag	na and the fathead minnow. Environmental
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Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Pimephales promelas; Embryo

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

Domain		Metric	Rating	Comments
Domain 1: Test Substa	ance			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	The chemical purity was reported as 99.5 %.
Domain 2: Test Desig	n			
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported for the preliminary test.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure (	haracterization			
Domain 5. Exposure v	Metric 7:	Experimental System/Test Media	Low	The study provided only limited details on the measures taken to appropriately prepare
	1,101110 / 1	Preparation	20	test concentrations.
	Metric 8:	Consistency of Exposure	Medium	Daily renewals occurred, but few details were provided.
		Administration		·
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure was reported but was shorter than recommended.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	wieure 11.	Spacing of Exposure Levels	mgn	response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organ	iem			
Domain 4. Test Organ	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms, but details
	Wietire 1 II	Conditions	111511	were limited.
	Metric 15:	Number of Organisms and	Medium	There were 30-50 embryos per treatment with two replicates.
		Replicates per Group		
Domain 5: Outcome A	Assessment			
Domain 3. Outcome 1	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism
	mente 10.	racquacy of rest conditions	111511	health.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1336024 Table: 1 of 1

### ... continued from previous page

Study Citation: Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Pimephales promelas*; Embryo

**Health Outcome:** 

Mortality

leatin Outcome: Mortanty

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

HERO ID:	1550024			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome	High	Outcomes were assessed consistently across study groups.
		Assessment		
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	<b>Explanation of Unexpected Outcomes</b>	Low	There was unexpectedly low survival in the negative control.

Additional Comments:

This form is for fathead minnow morality in the preliminary range finding test. Results were reported in the text as an LC50 value for 96h of 2.02mg/L for DBP. Confidence intervals (95%) were reported as well.

### **Overall Quality Determination**

### Medium

Study Citation:	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental
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Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Vertebrate; Fish; Pimephales promelas; Embryo

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

meno ie.	1550021				
Domain		Metric	Rating	Comments	
Domain 1: Test Substa	ance				
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only	
	Metric 2: Test Substance Source		Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.5 %	
Domain 2: Test Desig	n				
	Metric 4: Negative Controls Metric 5: Negative Control Response		High	Study authors reported using an appropriate concurrent negative control group	
			Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes although the solvent control had poor survival and hatch	
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure (	haracterization				
2 omain 5. Exposure (	Metric 7: Experimental System/Test Media		Low	The study provided only limited details on the measures taken to appropriately prepare	
		Preparation		test concentrations	
	Metric 8:	Consistency of Exposure	Medium	Daily renewals but few details were provided	
		Administration			
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured and are similar to nominal concentrations	
	Concentration Metric 10: Exposure Duration and Frequency		Medium	The duration of exposure was reported but was shorter than recommended	
	Metric 11:	1		The number of exposure groups and spacing of exposure levels were suitable for a dose	
	Wictic 11.	Spacing of Exposure Levels		response	
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit	
	Wietrie 12.	Testing at of Below Soldonity Emili	mgn	Exposure concentrations were at or below the water solubility limit	
Domain 4: Test Organ	ism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source	
	Metric 14:	Acclimatization and Pretreatment	High	Pretreatment conditions were the same for control and exposed organisms, details were	
		Conditions	C	limited	
	Metric 15:	Number of Organisms and	Medium	30-50 embryos per treatment with two replicates	
		Replicates per Group			
Domain 5: Outcome A	Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health	
		Conti	inued on next pa	age	
		Conti	mueu on next pa	ige	

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1336024 Table: 1 of 1

### ... continued from previous page

Study Citation: Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

Additional Comments: This form is for fathead minnow morality in the early life-stage test.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Pimephales promelas*; Embryo

Health Outcome:

Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

HERO ID:	1336024			
Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
	Metric 18:	Consistency of Outcome	High	outcomes were assessed consistently across study groups
		Assessment		
Domain 6: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically-significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
D : 7 D : D	1			
Domain 7: Data Pres				
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	Low	Unexpectedly low survival in negative control

**Overall Quality Determination** 

Medium

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Salmo mykiss*; Juvenile

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

1521776, Edited 11240 15(6). 1521776, 1515221								
Domain		Metric	Rating	Comments				
Domain 1: Test Subs								
	Metric 1:	Test Substance Identity	Low Low	The test substance nomenclature was reported without a CASRN.				
	Metric 2:	Metric 2: Test Substance Source		The test substance was provided by a manufacturer from commercially available				
				batches. The manufacture's name and batch number were not provided. No analytical				
	Metric 3:	Test Substance Purity	Ціah	data was reported.  The test substance was at least 95% pure.				
	Wietife 3.	Test Substance Furity	High	The test substance was at least 95% pure.				
Domain 2: Test Desi	ign							
	Metric 4:	Negative Controls	High	A negative control was reported.				
	Metric 5:	Negative Control Response	High	The control response was acceptable.				
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.				
Domain 3: Exposure Characterization  Metric 7: Experimental System/Test Media Medium The experimental system was well described. However, headspace or measures to pre-								
	Metric 7:	Metric 7: Experimental System/Test Media		The experimental system was well described. However, headspace or measures to pre-				
	M-4 0.	Preparation	TT: _1_	vent volatilization were not reported.				
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across groups.				
	Metric 9:	Measurement of Test Substance	High	Sample extracts were analyzed by gas chromatography at the start and end of the test.				
		Concentration						
	Metric 10:	Exposure Duration and Frequency	High	The duration and frequency of exposure were appropriate for the test.				
	Metric 11:	Number of Exposure Groups/	High	Exposure levels were appropriate. A range finding test was performed.				
	M-4-:- 10.	Spacing of Exposure Levels	TT: _1_					
	Metric 12:	Testing at or Below Solubility Limit	High	The test was performed at or below the water solubility limit.				
Domain 4: Test Orga	anism							
	Metric 13:	Test Organism Characteristics	Low	The source was not reported.				
	Metric 14:	Acclimatization and Pretreatment	High	Organisms were acclimated appropriately.				
		Conditions	_					
	Metric 15:	Number of Organisms and	Medium	Each test was performed using duplicate test concentrations with ten organisms per test				
		Replicates per Group		vessel.				
Domain 5: Outcome	Assessment							
Domain J. Outcome	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.				
				*				
		Cont	tinued on nex	κτ page				

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

### ... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Vertebrate; Fish; *Salmo mykiss*; Juvenile

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
	Metric 18: Consistency of Outcome		High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confound	ing / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Preso	entation and Anal	vsis		
Domain // Data 1100	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**

Study Citation: Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1332972; Linked HERO ID(s): 813673, 1332972

Domain Metric		Metric	Rating	Comments	
Domain 1: Test Substan	ce				
	Metric 1:	Test Substance Identity	Low	Nomenclature was referenced on page 13/62. No CAS or structure was listed.	
	Metric 2:	Test Substance Source	Low	Source was listed from Monsanto but not analytically verified.	
	Metric 3:	Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design					
C	Metric 4:	Negative Controls	Low	Ethanol was listed for solvent controls, however, authors reported using ethanol concentrations at higher than recommended for acute toxicity testing (1.8 ml/L) to increase solubility of compounds.	
	Metric 5:	Negative Control Response	Low	Control responses are not reported for acute toxicity bioassays.	
	Metric 6:	Randomized Allocation	Low	There was no reporting on how animals were allocated to treatment concentrations.	
Domain 3: Exposure Ch	aracterization				
•	Metric 7:	Experimental System/Test Media Preparation	Medium	The acute toxicity bioassays were conducted as static non-renewal, with morality 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 were reported as nominal.	
	Metric 10:	Exposure Duration and Frequency	High	The duration (48 hr) is appropriate.	
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	None of the treatment concentrations for the acute bioassays are reported. Range finding tests were not described.	
	Metric 12:	Testing at or Below Solubility Limit	Low	The LC50 values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since they were not reported.	
Domain 4: Test Organis	m				
	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia MO. The life stages were identified.	
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.	
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The authors listed 10 individuals per treatment concentrations but did not report the level of replication for each treatment.	

### Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1332972 Table: 1 of 2

### ... continued from previous page

**Study Citation:** Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1332972; Linked HERO ID(s): 813673, 1332972

Domain	Domain Metric  Metric 16: Adequacy of Test Conditions		Rating	Comments
			Medium	Dissolved oxygen, temperature, and photoperiod were reported for the acute bioassays.
	Metric 17:	Outcome Assessment Methodology	High	The authors had a very detailed list of multiple criteria to determine mortality from the bioassays.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes appear to be reported consistently.
Domain 6: Confounding	/ Variable Co	ntrol		
	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 Presenta	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Authors used Litchfield and Wilcoxon method for LC50 estimation.
	Metric 22:	Reporting of Data	Medium	LC 50 and 95% confidence intervals are reported, but results from each concentration are not available.
	Metric 23:	<b>Explanation of Unexpected Outcomes</b>	High	No unexpected outcomes were reported.

Additional Comments: None

## **Overall Quality Determination**

## Medium

Study Citation: Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; *Chironomus plumosus*; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1332972; Linked HERO ID(s): 813673, 1332972

Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Source Metric 3: Test Substance Purity  Domain 2: Test Design Metric 4: Negative Controls Metric 5: Negative Controls Metric 6: Randomized Allocation  Metric 6: Randomized Allocation  Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Metric 9: Measurement of Test Substance Concentration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Groups/ Metric 11: Number of Exposure Groups/ Metric 12: Test Organism  Metric 13: Test Organism  Metric 14: Acclimatization and Pretreatment Conditions  Metric 14: Test Substance Low Source on page 13/62. No CAS or structure listed. No purity reported. No purity reported for acute toxicity bioassays. No reporting on how animals were conducted as static non-renewal, with morality assessed at 24 and 48 hours.  Ethanol listed for solvent controls, however, authors report using ethanol concentrations.  Medium The acute toxicity bioassays are reported on treatment concentrations were conducted as static non-renewal, with morality assessed at 24 and 48 hours.  Ethanol listed for solvent controls, however, authors report using ethanol concentrations were conducted as static non-renewal, with morality assessed at 24 and 48 hours.  Ethanol listed for solvent controls, however, authors report using ethanol concentration at higher than recommended for acute toxicity bioassays are reported on the function of the function of the function of the function of the fun	HERO ID:	13329/2; Linked HERO ID(s): 8136/3, 13329/2								
Metric 1: Test Substance Identity Metric 2: Test Substance Source Metric 3: Test Substance Source Metric 3: Test Substance Source Metric 3: Test Substance Purity  Domain 2: Test Design  Metric 4: Negative Controls  Metric 5: Negative Controls  Metric 6: Randomized Allocation  Metric 6: Randomized Allocation  Metric 7: Experimental System/Test Media Preparation  Metric 8: Consistency of Exposure  Metric 9: Measurement of Test Substance Concentration  Metric 10: Exposure Torontory  Metric 11: Number of Exposure Levels  Metric 12: Testing at or Below Solubility Limit  Metric 12: Testing and Pretreatment  Metric 13: Test Organism  Metric 14: Acclimatization and Pretreatment  Metric 15: Number of Organisms and  Metric 15: Number of Organisms and  Metric 15: Number of Organisms and  Metric 16: Test Substance Conditions  Metric 17: Test Substance Conditions  Metric 18: Test Organism Conditions  Metric 19: Testing at or Below Solubility Limit  Metric 19: Testing at or Below Solubility Limit  Metric 10: Testing and pretreatment Concentrations were under the solubility reported as from the CNFRL in Columbia MO. The life stages were identified.  Metric 19: Testing and pretreatment  Metric 19: Testing and pretreatment  Metric 19: Testing at or Below Solubility Limit  Metric 19: Testing at or Below Solubility Limit  Metric 10: Testing at or Below Solubility Limit  Metric 11: Test Organism  Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism Characteristics  Metric 14: Acclimatization and Pretreatment  Metric 15: Number of Organisms and  Low The authors listed 10 individuals per treatment concentrations but did not report the level of the author of the subscience of	Domain		Metric	Rating	Comments					
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Metric 3: Test Substance Purity  Low No purity reported.  Domain 2: Test Design  Metric 4: Negative Controls  Metric 5: Negative Control Response Metric 6: Randomized Allocation  Domain 3: Exposure Characterization Metric 7: Experimental System/Test Media Preparation Metric 8: Consistency of Exposure Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Genous/ Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism  Metric 14: Acclimatization and Pretreatment Metric 15: Negative Control Response  Low Control responses are not reported for acute toxicity bioassays.  Medium The acute toxicity bioassays were conducted as static non-renewal, with morality assessed at 24 and 48 hours.  exposure administration appeared consistent among treatments and control.  Administration Metric 10: Exposure Duration and Frequency Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism  Metric 14: Acclimatization and Pretreatment  Metric 15: Negative Controls  Metric 16: Negative Control Response  Low Non of the treatment concentrations for the acute bioassays are reported. Range finding tests were not described.  The LCSO values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since the were not reported.  Domain 4: Test Organism  Metric 13: Test Organism Characteristics  Metric 14: Acclimatization and Pretreatment  Conditions  Metric 15: Number of Organisms and  Low The authors listed for solvent controls, however, authors require toxicity tests.  The pretreatment conditions were issed and similar to the 48 hr acute toxicity tests.  The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.  The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.  The pretreatment conditions were listed and similar to		Metric 1:	Test Substance Identity	Low	Nomenclature referenced on page 13/62. No CAS or structure listed.					
Metric 4: Negative Controls  Low Ethanol listed for solvent controls, however, authors report using ethanol concentration at higher than recommended for acute toxicity testing (1.8 ml/L) to increase solubility compounds.  Metric 5: Negative Control Response Low Control responses are not reported for acute toxicity bioassays.  Metric 6: Randomized Allocation  Metric 7: Experimental System/Test Media Preparation  Metric 8: Consistency of Exposure  Metric 8: Consistency of Exposure  Metric 9: Measurement of Test Substance Concentration  Metric 10: Exposure Duration and Frequency  Metric 11: Number of Exposure Facus are not reported on seven to analyzed and LC50 concentrations are reported as nominal.  Metric 12: Testing at or Below Solubility Limit  Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism  Metric 14: Acclimatization and Pretreatment  Metric 14: Acclimatization and Pretreatment  Conditions  Metric 15: Number of Grganisms and  Low The acute bioassay concentrations were not analyzed and LC50 concentrations are reported as nominal.  Non of the treatment concentrations for the acute bioassays are reported. Range finding tests were not described.  The LC50 values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since the were not reported.  Metric 14: Acclimatization and Pretreatment  Conditions  Metric 15: Number of Organisms and  Low The authors listed 10 individuals per treatment concentrations but did not report the levent and the solubility of the preparation and perton tests.		Metric 2: Test Substance Source		Low	Source was listed from Monsanto but not analytically verified.					
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Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels  Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism  Metric 13: Test Organism Characteristics  Metric 14: Acclimatization and Pretreatment  Conditions  Metric 15: Number of Organisms and  Metric 15: Number of Organisms and  Non of the treatment concentrations for the acute bioassays are reported. Range finding tests were not described.  Low The LC50 values are under the solubility reported in the Final Scope for DBP (11.2 mg/L). It is not certain if the range of concentrations were under the solubility since the were not reported.  The source of animals was reported as from the CNFRL in Columbia Mo. The life stages were identified.  Metric 14: Acclimatization and Pretreatment  Conditions  Number of Organisms and  Low The authors listed 10 individuals per treatment concentrations but did not report the level of the stages were identified.		Metric 9:	Measurement of Test Substance	Low						
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Metric 15: Number of Organisms and Low The authors listed 10 individuals per treatment concentrations but did not report the lev		Metric 14:		High	The pretreatment conditions were listed and similar to the 48 hr acute toxicity tests.					
		Metric 15:	Number of Organisms and	Low	The authors listed 10 individuals per treatment concentrations but did not report the leve of replication for each treatment.					
Jamein 5, Outcome Aggessment	Domain 5. Outage - A	agagamant								
Oomain 5: Outcome Assessment  Metric 16: Adequacy of Test Conditions  Medium  DO, temperature, and photoperiod were reported for the acute bioassays.	Domain 5: Outcome A		Adequacy of Test Conditions	Medium	DO, temperature, and photoperiod were reported for the acute bioassays.					
Continued on next page				nued on next no						

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1332972 Table: 2 of 2

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Study Citation: Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Aquat Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 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: Confoundi	ng / Variable Co	ntrol		
	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	No information to suggest differences among groups related to health outcomes.
Domain 7: Data Prese	ntation and Anal	lysis		
	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

Stud	v Citation:	Streufort, J. M.	(1978)	). Some effects of two	phthalic acid esters on the life of	cycle of the midge	(Chironomus plumosus).
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**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1332972; Linked HERO ID(s): 813673, 1332972

Domain	Metric		Rating	Comments
Domain 1: Test Substa	ance			
	Metric 1:	,		Nomenclature was referenced on page 13/62. No CAS or structure were listed.
	Metric 2:	Test Substance Source	Low	Source was listed from Monsanto but not analytically verified.
	Metric 3:	Test Substance Purity	Low	No purity was reported.
Domain 2: Test Desig	n			
	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			
Zonam er Zapesare	Metric 7:	Experimental System/Test Media Preparation	High	The chronic exposure were conducted with a flow-through system for continual renewal of the chemical. The setup and flow-rate was described well on page 32/62.
	Metric 8:	Consistency of Exposure	High	Exposure administration appeared consistent among treatments and control.
	Metric 9:	Administration Measurement of Test Substance	Medium	GC was used to verify the concentrations from the chronic exposure on page 25/62.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The chronic exposures for midge emergence ranged from 20-40 days for hydrosoil 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 all below the published solubility value published in the final scope for DBP (11.2 mg/l).
Domain 4: Test Organ	nism			
Domain 1. Test Organ	Metric 13:	Test Organism Characteristics	High	The source of animals was reported as from the CNFRL in Columbia, MO. The life stages were identified.
	Metric 14:	Acclimatization and Pretreatment	High	The pretreatment conditions were listed and similar to the chronic exposures.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	Authors began each treatment and control group with 100 1st instar larvae for the chronic emergence bioassay. The replication and housing groups for this work was not well described.

#### Domain 5: Outcome Assessment

### Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1332972 Table: 1 of 1

### ... continued from previous page

Study Citation: Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).

**Duration:** Overall Duration: > 21 days; Exposure Duration: > 21 days

Exposure Route, Amedia, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus plumosus; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1332972; Linked HERO ID(s): 813673, 1332972

Domain		Metric		Comments
	Metric 16:	Adequacy of Test Conditions	Medium	Dissolved oxygen, temperature, and photoperiod were reported for the chronic expo-
				sures.
	Metric 17:	Outcome Assessment Methodology	Medium	No significant differences in emergence were observed for the chronic exposures.
	Metric 18:	Consistency of Outcome	High	The outcomes appear to be reported consistently.
		Assessment		
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	Nothing was reported to indicate differences among treatment groups with environmen-
		Design and Procedures		tal conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups related to health out-
				comes.
Domain 7: Data Present	estion and Anal	, voic		
Domain 7. Data Fresent		•	TT' 1	
	Metric 21:	Statistical Methods	High	The percent data was arc sin square-root transformed and Least Significant Difference tests were performed.
	Metric 22:	Reporting of Data	Medium	Emergence is reported in total numbers for each concentration, compound, and day of exposure in tables presented in the results section.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

# **Overall Quality Determination**

## Medium

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,
	Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment
	exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.
D4!	One of Department of the form

**Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Media, Path: Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Chironomus tentans; Larvae

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 679311

Domain		Metric	Rating	Comments
Domain 1: Test Subst	ance			
	Metric 1:	Test Substance Identity	High	Chemical was identified as a "single isomer" and the identify, including CASRN referenced in an outside paper, Call et al 2001.
	Metric 2:	Test Substance Source	High	Source of chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	Purity was identified as >99% in Call et al 2001.
Domain 2: Test Desig	n			
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	Biological response of control groups was appropriate as shown in Table 4.
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.
Domain 3: Exposure (	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 reported and consistent among different DBP treatments and controls.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured using HPLC as described in methods and cited reference (Call et al 2001).
	Metric 10:	Exposure Duration and Frequency	High	Duration (10 day exposure) was appropriate for experimental design.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were acceptable and spanned 5 concentrations per test species in addition to control; nominal doses unclear however measured doses reported in sediment and pore water.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via sediment.
Domain 4: Test Organ	nism			
C	Metric 13:	Test Organism Characteristics	Low	Source of test organism not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimation of test organisms prior to exposure not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized 3 replicates of five different concentrations with 10 organisms per beaker and three sediment control replicates with 10 test organisms per beaker and three silica sand control replicates with 10 test organisms per beaker.

### ... continued from previous page

Study Citation: Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,

Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment

HERO ID: 679311 Table: 1 of 2

exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.

**Duration:** 

Chemical:

Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Arthropods; Chironomus tentans; Larvae

**Health Outcome:** 

**Dutcome:** Mortality

Diisobutyl Phthalate- Parent compound

**HERO ID:** 679311

Domain		Metric	Rating	Comments
Domain 5: Outcome Asse	essment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions described in Table 2.
	Metric 17:	Outcome Assessment Methodology	Medium	Survivor count determined after the 10 day exposure but not reported as percent mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted at conclusion of 10 day exposure.
Domain 6: Confounding	Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	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 Presentat	tion and Anal	vsis		
	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman–Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStatt Program.
	Metric 22:	Reporting of Data	High	Survival data reported in Table 4 and LC50 values shown in Figure 1 and Table 6.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability not reported but results suggest no excessive variability within replicates.

### **Overall Quality Determination**

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,
	Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment
<b>5</b>	exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.

**Duration:** Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route, Media, Path:

Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Invertebrate; Arthropods; Chironomus tentans; Larvae

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 679311

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	High	The chemical was identified as a "single isomer" and the identify, including CASRN referenced in an outside paper, Call et al 2001.
	Metric 2:	Test Substance Source	High	The source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	The purity was identified as >99% in Call et al 2001.
Domain 2: Test Design				
Č	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	Biological response of control groups was appropriate as shown in Table 4.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Evnosura C	noracterization			
Domain 3: Exposure Cl	Metric 7:	Experimental System/Test Media	High	Methods of sediment collection and preparation (including chemical addition) and addi-
	Michie 7.	Preparation	111511	tion of sediment to test beakers were described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure consistency was reported and consistent among different DBP treatments and controls.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in the methods and cited reference (Call et al 2001).
	Metric 10:	Exposure Duration and Frequency	High	The duration (10-day exposure) was appropriate for the experimental design.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were acceptable and spanned five concentrations per test species in addition to the control. Nominal doses were unclear, however measured doses were reported in sediment and pore water.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via sediment.
Domain 4: Test Organis	sm			
	Metric 13:	Test Organism Characteristics	Low	The source of test organisms was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to exposure was not reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized three replicates of five different concentrations with 10 organisms per beaker; three sediment control replicates with 10 test organisms per beaker; and three silica sand control replicates with 10 test organisms per beaker.

### Domain 5: Outcome Assessment

### Continued on next page ...

HERO ID: 679311 Table: 2 of 2

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Study Citation:  Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	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. exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.  Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days  Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact upto dia, Path:  (a, Species, Age: alth Outcome: Invertebrate; Arthropods; Chironomus tentans; Larvae  Development/Growth				
HERO ID:	679311				
Domain		Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions were described in Table 2.	
	Metric 17:	Outcome Assessment Methodology	High	Sediment was sieved, survivors collected, dried, and weighed.	
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was conducted at the conclusion of the 10-day exposure.	
Domain 6: Confounding	/ Variable Co	ntrol			
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.	
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group, and there were no differences among groups that could influence the outcome assessment.	
Domain 7: Data Presenta	ation and Anal	vsis			
	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman–Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStatt Program.	
	Metric 22:	Reporting of Data	High	Treatment and control data were reported in Table 4. Results were represented as average dry weight per individual.	
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported, but results suggest no excessive variability within replicates.	
Additional Comments:	None				

**Overall Quality Determination** 

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Daphnia magna; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** In

Immobilization

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported as provided by a manufacturer from commercially available batches. The manufacture name and batch number were not provided. No analytical data were reported.
	Metric 3:	Test Substance Purity	High	The substance was at least 95% pure.
Domain 2: Test Design	1			
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.
Domain 3: Exposure C	haracterization			
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure	High	The exposure administration was consistent across groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Sample extracts were analyzed by gas chromatography at the start and end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ 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 water solubility.
Domain 4: Test Organi	sm			
<i>8</i>	Metric 13:	Test Organism Characteristics	Low	The source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for the test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.

### Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

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Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Invertebrate; Arthropods; *Daphnia magna*; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Immobilization

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 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	High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confound	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistent across groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Preso	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental
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Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** Mortality

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

Domain		Metric	Rating	Comments
Domain 1: Test Substa	ance		-	
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.5 %.
Domain 2: Test Design	n			
_	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	High	No mortalities were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	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	No details were provided.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured after Day 1 and reported in Table 2.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	wieure 11.	Spacing of Exposure Levels	Tilgii	response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Most exposure concentrations were below the water solubility limit. The high concentration was close.
Domain 4: Test Organ	iom			
Domain 4. Test Organ	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
				"Test animals for both the acute immobilization tests (range finding test) and the reproduction test were collected when the animals were less than 24 h old."
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Pretreatment conditions were the same for control and exposed organisms, but details were limited.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were five daphnids per treatment with two replicates.
Damain 5. Out				
Domain 5: Outcome A	Assessment Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions were not sufficiently reported to evaluate if adequate.
			nued on next pa	

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1336024 Table: 1 of 1

#### ... continued from previous page

Study Citation: Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Daphnia magna; Juvenile

Health Outcome:

Mortality

Chemical: Mortant

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology was reported in the text.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group in text form.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments:

Range finding test:"In the acute mortality test (range-finding test), all D. magna were dead after 48 h of exposure to nominal concentrations of 7.5 and 10.0 mg/L DBP. At the lower doses of 3.0, 1.0 and 0.5 mg/L DBP and in controls, all animals survived, except for one individual at 3.0 mg/L. The LC50 (lethal concentration to 50% of the test population) is between 3.0 and 7.5 mg/L DBP. Although a probit analysis cannot be performed, because this procedure requires two responses that are between 0 and 100% mortality, a nonparametric analysis was developed for steep dose-response bioassays (Schmoyer, Beauchamp and McCarthy, manuscript in preparation). The LC50 was estimated using this method and was equal to 5.2 mg/L, with 95% confidence limits of 4.7 and 5.6 mg/L."

### **Overall Quality Determination**

Study Citation:	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia
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magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) **Duration:** 

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** 

Mortality

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 4829279

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.
	Metric 5:	Negative Control Response	High	Survival in experimental controls and vehicle controls was 100%.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Ch	aracterization			
Domain J. Exposure Cir	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. Exposure was conducted in 100mL glass beakers.
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.
Domain 4: Test Organis	m			
C	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but culture origin 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 (10 organisms per treatment per beaker and replicated five times).

#### Domain 5: Outcome Assessment

#### Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation

#### ... continued from previous page

Study Citation: Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 4829279 Table: 1 of 1

Media, Path:

**Taxa, Species, Age:** Invertebrate; Arthropods; *Daphnia magna*; Juvenile

**Health Outcome:** 

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 4829279

Domain		Metric	Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confoundi	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text and tables.
	Metric 23:	<b>Explanation of Unexpected Outcomes</b>	High	There were no unexpected outcomes.

Additional Comments: None

**Overall Quality Determination** 

Study Citation: Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

Domain		Metric	Rating	Comments
Domain 1: Test Subst	tance			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.5 %.
Domain 2: Test Desig	gn			
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes, although the non-solvent control had poor survival and fewer young.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure	Characterization			
Domain 3. Exposure	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations
	Metric 8:	Consistency of Exposure	Medium	Daily renewals but few details were provided
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured but are considerably lower than nominal concentrations
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit
Damain 4. Test Organ	niam.			
Domain 4: Test Organ	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source. "Test animals for both the acute immobilization tests (range finding test) and the reproduction test were collected when the animals were less than 24 h old."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms, details were limited
	Metric 15:	Number of Organisms and Replicates per Group	Low	40 daphnids per treatment but no replicates

#### Domain 5: Outcome Assessment

Diisobutyl Phthalate **Environmental Hazard Evaluation** HERO ID: 1336024 Table: 1 of 3

#### ... continued from previous page

**Study Citation:** Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

HERO ID: 1336024

пекотр:	1330024			
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: Confoundin	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statisticall significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the sam problem was evident in the DOP experiment (see below). By the end of the experimen (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Presen	tation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control grou
	Metric 23:	Explanation of Unexpected Outcomes	Low	Unexpectedly low survival in clean control

organisms in this study as well as number of young per adult. The study authors attempted to bypass the survivability issue relate results to number of young per adult, but with such variability in controls introduces uncertainty. The solvent control should be used as an alternative.

### **Overall Quality Determination**

HERO ID: 1336024 Table: 2 of 3

**Study Citation:** Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days **Duration:** 

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** 

Mortality

**Chemical:** Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

HERO ID.	100002.			
Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as 99.5 %
Domain 2: Test Design	n			
_	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes although the non-solvent control had poor survival and fewer young
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure (	Characterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations
	Metric 8:	Consistency of Exposure	Medium	Daily renewals but few details were provided
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured but are considerably lower than nominal concentrations
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit
Domain 4: Test Organ	ism			
Domain 1. Test Organ	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source "Test animals for both the acute immobilization tests (range finding test) and the reproduction test were collected when the animals were less than 24 h old."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms, details were limited
	Metric 15:	Number of Organisms and Replicates per Group	Low	40 daphnids per treatment but no replicates
Domain 5: Outcome A	Assessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of test system were conducive to maintenance of organism health

Diisobutyl Phthalate **Environmental Hazard Evaluation** HERO ID: 1336024 Table: 2 of 3

#### ... continued from previous page

Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental **Study Citation:** 

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Juvenile

**Health Outcome:** 

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

HERO ID: 1336024

HERO ID.	1330024			
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: Confoundi	ing / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically-significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups
Domain 7: Data Prese	entation and Anal	vsis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group
	Metric 23:	Explanation of Unexpected Outcomes	Low	Unexpectedly low survival in clean control

Additional Comments:

This form is to account for mortality in chronic Daphnia magna test with results found in Table 3. There were serious concerns regarding the survivability of negative control organisms in this study as well as number of young per adult. The study authors attempted to bypass the survivability issue relate results to number of young per adult, but with such variability in controls introduces uncertainty. The solvent control should be used as an alternative.

# **Overall Quality Determination**

**Study Citation:** Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days **Duration:** 

**Exposure Route,** 

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Invertebrate; Arthropods; Daphnia magna; Juvenile Taxa, Species, Age:

**Health Outcome:** 

Reproductive/Teratogenic

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 1336024

HERO ID.				
Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3:	Test Substance Purity	High	Chemical purity was reported as 99.5 %.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Medium	The biological response of the negative control group was reported and reasonable for assessed outcomes although the non-solvent control had poor survival and fewer young.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	haracterization			
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations.
	Metric 8:	Consistency of Exposure	Medium	Daily renewals occurred, but few details were provided.
	Metric 9:	Administration Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured, but are considerably lower than nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organi	sm			
Domain 1. 16st Organi	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source "Test animals for both the acute immobilization tests (range finding test) and the reproduction test were collected when the animals were less than 24 h old."
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for control and exposed organisms, but details were limited.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 40 daphnids per treatment, but no replicates used.
Domain 5: Outcome A	ssessment			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1336024 Table: 3 of 3

#### ... continued from previous page

Study Citation: Mccarthy, J. F., Whitmore, D. K. (1985). Chronic toxicity of di-n-butyl and di-n-octyl phthalate to daphnia-magna and the fathead minnow. Environmental

Toxicology and Chemistry 4(2):167-179.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

**Taxa, Species, Age:** Invertebrate; Arthropods; *Daphnia magna*; Juvenile

**Health Outcome:** 

Reproductive/Teratogenic

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1336024

HERO ID.	1330024			
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: Confound	ding / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Results for this criterion should be related to the solvent control. There was statistically-significant morality in the negative control group that may impact results for reproductive effects. The study authors attempted to salvage the study by relating everything to surviving D. magna. However, this introduces further uncertainty regarding the actual effects of DBP on D. magna. "Survival of D. magna exposed to DBP exceeded 80% in all concentrations except 1.8 and 3.2 mg/L and in the carrier-free control. The reason for the poor survival (and poor reproduction) of the control group is not clear; the same problem was evident in the DOP experiment (see below). By the end of the experiment (day 16),70% of the D. magna were alive at 1.8 mg/L and 18% were alive at 3.2 mg/L DBP (Fig. 1 and Table 3)."
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	Low	There was unexpectedly low survival in the clean control.
		·		

Additional Comments:

This form is for D. manga reproductive effects - total broods and days to primiparous instar. There were serious concerns regarding the survivability of negative control organisms in this study as well as number of young per adult. The study authors attempted to bypass the survivability issue by relating results to number of young per adult, but with such variability in controls it introduces uncertainty. The solvent control should be used as an alternative.

# **Overall Quality Determination**

Study Citation:	Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia
	magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Aquatic (freshwater); Water; Not determined by study authors (i

Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Daphnia magna; Adult

**Health Outcome:** Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 4829279

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	Low	Chemical was identified by name only
	Metric 2:	Test Substance Source	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.
	Metric 3:	Test Substance Purity	High	Chemical purity reported as >99%
Domain 2: Test Design				
Č	Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups
	Metric 5:	Negative Control Response	High	survival in experimental controls and vehicle controls was 100%.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
D : 0 E G				
Domain 3: Exposure Ch				
	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The exposure was conducted with individual neonates in 20mL glass tubes.
	Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not administered consistently across study groups
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate
D : 4 T : 0 :				
Domain 4: Test Organis		T+ O	M - J:	
	Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, culture origin 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 (15 organisms per treatment, in individual 20mL glass tubes).

#### Domain 5: Outcome Assessment

#### Continued on next page ...

Diisobutyl Phthalate Environmental Hazard Evaluation

#### ... continued from previous page

Study Citation: Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

HERO ID: 4829279 Table: 1 of 2

Taxa, Species, Age:

**Age:** Invertebrate; Arthropods; *Daphnia magna*; Adult

**Health Outcome:** 

Reproductive/Teratogenic

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 4829279

Domain		Metric	Rating	Comments
Me	etric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported
Me	etric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest
Me	etric 18:	Consistency of Outcome Assessment	High	outcomes were assessed consistently across study groups
Domain 6: Confounding / Var	riable Cor	ntrol		
Me	etric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions
Me	etric 20:	Outcomes Unrelated to Exposure	High	there were no differences among groups
Domain 7: Data Presentation	and Analy	ysis		
Me	etric 21:	Statistical Methods	High	Statistical methods were adequately described
Me	etric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text and tables
Me	etric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

Additional Comments: generational effects also reported

**Overall Quality Determination** 

HERO ID: 4829279 Table: 2 of 2

Study Citation: Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia

magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Adult

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 4829279

		Rating	
e			
Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
Metric 2:	Test Substance Source	High	The test substance was obtained from S&T Ltd, Tianjin, China. The test substance was identified by GC-MS.
Metric 3:	Test Substance Purity	High	Chemical purity was reported as >99%.
Metric 4:	Negative Controls	High	Study authors reported using appropriate concurrent negative control groups.
Metric 5:	Negative Control Response	High	Survival in experimental controls and vehicle controls was 100%.
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
racterization			
Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. The exposure was conducted with individual neonates in 20mL glass tubes.
Metric 8:	Consistency of Exposure Administration	Medium	Nothing in the study suggested that exposures were not administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was appropriate.
1			
Metric 13:	Test Organism Characteristics	Medium	The test organisms were adequately described, but culture origin was not reported.
Metric 14:	Acclimatization and Pretreatment	High	All pretreatment conditions were the same for control and exposed organisms.
Metric 15:	Number of Organisms and	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects (15 organisms per treatment, in individual 20mL glass tubes).
	Trephenies per Group		C 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Metric 16:	Adequacy of Test Conditions	Medium	Environmental conditions of the test system were most likely conducive to maintenance of organism health but actual measured condition values for control and exposed vessels was not reported.
	Metric 2:  Metric 3:  Metric 4: Metric 5: Metric 6:  Metric 6:  Metric 7:  Metric 8:  Metric 9:  Metric 10:  Metric 11:  Metric 12:  Metric 12:  Metric 13:  Metric 14:  Metric 15:	Metric 2: Test Substance Source  Metric 3: Test Substance Purity  Metric 4: Negative Controls Metric 5: Negative Control Response Metric 6: Randomized Allocation  Metric 7: Experimental System/Test Media Preparation  Metric 8: Consistency of Exposure Administration Metric 9: Measurement of Test Substance Concentration Metric 10: Exposure Duration and Frequency  Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels Metric 12: Testing at or Below Solubility Limit  Metric 13: Test Organism Characteristics Metric 14: Acclimatization and Pretreatment Conditions Metric 15: Number of Organisms and Replicates per Group  essment Metric 16: Adequacy of Test Conditions	Metric 2: Test Substance Source High  Metric 3: Test Substance Purity High  Metric 4: Negative Controls High  Metric 5: Negative Control Response High  Metric 6: Randomized Allocation Low  aracterization  Metric 7: Experimental System/Test Media Preparation  Metric 8: Consistency of Exposure Medium Administration  Metric 9: Measurement of Test Substance High  Concentration  Metric 10: Exposure Duration and Frequency High  Metric 11: Number of Exposure Groups/  Spacing of Exposure Levels  Metric 12: Testing at or Below Solubility Limit High  Metric 13: Test Organism Characteristics Medium  Metric 14: Acclimatization and Pretreatment High  Conditions  Metric 15: Number of Organisms and Replicates per Group

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 4829279 Table: 2 of 2

#### ... continued from previous page

Study Citation: Wei, J., Shen, Q., Ban, Y., Wang, Y., Shen, C., Wang, T., Zhao, W., Xie, X. (2018). Characterization of Acute and Chronic Toxicity of DBP to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 101(2):214-221.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route,

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Daphnia magna; Adult

**Health Outcome:** 

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 4829279

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no differences among groups.
Domain 7: Data Present	ation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in the text and tables.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	molting freq	uency, generational effects also reported		

### **Overall Quality Determination**

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,
	Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment
	exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.
Duration	Overall Duration: $A = 10$ days: Evposure Duration: $A = 10$ days

Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

**Exposure Route,** Media, Path:

Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** 

Development/Growth

Chemical: **HERO ID:**  Diisobutyl Phthalate- Parent compound

679311

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	Metric 1:	Test Substance Identity	High	The chemical was identified as a "single isomer" and the identify, including CASRN was referenced in an outside paper, Call et al 2001.
	Metric 2:	Test Substance Source	High	Source of the chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	Purity was identified as >99% in Call et al 2001.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	Biological response of control groups was appropriate as shown in Table 4.
	Metric 6:	Randomized Allocation	Low	Random allocation was not reported.
Domain 3: Exposure Ch	aracterization			
•	Metric 7:	Experimental System/Test Media Preparation	High	Methods of sediment collection and preparation (including chemical addition) and addition of sediment to test beakers were described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure consistency was reported and consistent among different DBP treatments and controls.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations were measured using HPLC as described in methods and the cited reference (Call et al 2001).
	Metric 10:	Exposure Duration and Frequency	High	The 10-day exposure duration was appropriate for the experimental design.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Exposure groups were acceptable and spanned five concentrations per test species in addition to the control. Nominal doses were unclear, however measured doses were reported in sediment and pore water.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure was via sediment.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	Source of test organisms was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to the exposure was not reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Tests with DBP utilized three replicates of five different concentrations with 10 organisms per beaker, three sediment control replicates with 10 test organisms per beaker, and three silica sand control replicates with 10 test organisms per beaker.

Diisobutyl Phthalate Environmental Hazard Evaluation

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Study Citation: Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,

Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment

HERO ID: 679311 Table: 1 of 2

exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.

**Duration:** 

Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** 

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 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, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions were described in Table 2.
Metric 17:	Outcome Assessment Methodology	High	Sediment was sieved, survivors were collected, dried, and weighed.
Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was conducted at conclusion of the 10-day exposure.
Domain 6: Confounding / Variable Co	ntrol		
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Ana	lysis		
Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman-Karber method. Dry weight datawere analyzed by one-way analysis of variance and Dunnett's procedure using a SigmaStatt Program.
Metric 22:	Reporting of Data	High	Treatment and control data were reported in Table 4. Results were represented as average dry weight per individual.
Metric 23:	Explanation of Unexpected Outcomes	Medium	Variability was not reported, but results suggest no excessive variability within repli- cates.

Additional Comments: None

# **Overall Quality Determination**

Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A.,
	Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment
	exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.
<b>Duration:</b>	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days

Exposure Route,

Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Chemical:

Invertebrate; Arthropods; Hyalella azteca; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Mortality

Diisobutyl Phthalate- Parent compound

**HERO ID:** 679311

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	High	Chemical was identified as a "single isomer" and the identify, including CASRN referenced in an outside paper, Call et al 2001.
	Metric 2:	Test Substance Source	High	Source of chemical was defined in Call et al 2001 as AldrichChemical (Milwaukee, WI, USA).
	Metric 3:	Test Substance Purity	High	Purity was identified as >99% in Call et al 2001.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls (sediment and silica sand) were used in this experiment.
	Metric 5:	Negative Control Response	High	Biological response of control groups was appropriate as shown in Table 4.
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.
Domain 3: Evnosura Ch	agrantarization			
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	High	Methods of sediment collection and preparation (including chemical addition) and addi-
	wienie /.	Preparation	mgn	tion of sediment to test beakers were described in detail.
	Metric 8:	Consistency of Exposure	High	Exposure consistency reported and consistent among different DBP treatments and
		Administration	6	controls.
	Metric 9:	Measurement of Test Substance	High	Concentrations measured using HPLC as described in methods and cited reference (Call
		Concentration	-	et al 2001).
	Metric 10:	Exposure Duration and Frequency	High	Duration (10 day exposure) was appropriate for experimental design.
	Metric 11:	Number of Exposure Groups/	Medium	Exposure groups were acceptable and spanned 5 concentrations per test species in addi-
		Spacing of Exposure Levels		tion to control; nominal doses unclear however measured doses reported in sediment and pore water.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via sediment.
Domain 4: Test Organis	sm			
Č	Metric 13:	Test Organism Characteristics	Low	Source of test organism not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	Acclimation of test organisms prior to exposure not reported.
		Conditions		
	Metric 15:	Number of Organisms and	Medium	Tests with DBP utilized 3 replicates of five different concentrations with 10 organisms
		Replicates per Group		per beaker and three sediment control replicates with 10 test organisms per beaker and three silica sand control replicates with 10 test organisms per beaker.

#### Domain 5: Outcome Assessment

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HERO ID: 679311 Table: 2 of 2

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Study Citation:	Call, D. J., Cox, D. A., Geiger, D. L., Genisot, K. I., Markee, T. P., Brooke, L. T., Polkinghorne, C. N., Vandeventer, F. A., Gorsuch, J. W., Robillard, K. A., Parkerton, T. F., Reiley, M. C., Ankley, G. T., Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 2. Sediment exposures. Environmental Toxicology and Chemistry 20(8):1805-1815.						
<b>Duration:</b>		ation: 4 - 10 days; Exposure Duration: 4 -					
<b>Exposure Route,</b>	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route						
Media, Path:							
Taxa, Species, Age:	Invertebrate	; Arthropods; Hyalella azteca; Not Applica	able (e.g., fun	gi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mortality						
Chemical: HERO ID:	Diisobutyl P 679311	hthalate- Parent compound					
Domain		Metric	Rating	Comments			
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions including feeding, intermittent water renewal system, lighting, and temperature were reported. Authors recorded temperature, dissolved oxygen, and pH on days 0 and 10 and conductivity, hardness, alkalinity, and ammonia on days 1 and 9. Sediment TOC conditions described in Table 2.			
	Metric 17:	Outcome Assessment Methodology	Medium	Survivor count determined after the 10 day exposure but not reported as percent mortality.			
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted at conclusion of 10 day exposure.			
Domain 6: Confoundin	g / Variable Co	ntrol					
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.			
	Metric 20:	Outcomes Unrelated to Exposure	High	Details regarding test organism attrition and outcomes unrelated to exposure were re- ported for each study group and there were no differences among groups that could influence the outcome assessment.			
Domain 7: Data Presen	ntation and Anal	lycic .					
Domain 7. Data Hesen	Metric 21:	Statistical Methods	High	Survival data from toxicity tests were summarized using the trimmed Spearman–Karber			
	Medic 21.	Statistical Methods	mgn	method. Dry weight datawere analyzed by one-way analysis of variance and Dunnett'sprocedure using a SigmaStatt Program.			
	Metric 22:	Reporting of Data	High	Survival data reported in Table 4 and LC50 values shown in Figure 1 and Table 6.			
	Metric 23:	<b>Explanation of Unexpected Outcomes</b>	Medium	Variability not reported but results suggest no excessive variability within replicates.			

Additional Comments: None

# **Overall Quality Determination**

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
	: F : (1FF : 1 1G) : (14/0) 15/0 1574

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Paratanytarsus parthenogenetica; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Mortality

**Chemical:** Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substan	ice			
	Metric 1:	Test Substance Identity	Low	Test substance nomenclature was reported without CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported as provided by manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	The chemical was at least 95% pure.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.
Domain 3: Exposure Ch	naracterization			
Domain 3. Exposure of	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	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 end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure were appropriate for this test.
	Metric 11:	Number of Exposure Groups/ 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 water solubility.
Domain 4: Test Organis	m			
	Metric 13:	Test Organism Characteristics	Low	A source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation for the test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.

#### Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

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Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Paratanytarsus parthenogenetica; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** 

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 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	High	Outcome assessment was consistent across groups.
	Assessment		
Domain 6: Confounding / Variabl	e Control		
Metric	<ol><li>Confounding Variables in Test</li></ol>	High	Environmental conditions were consistant across groups.
	Design and Procedures		
Metric :	20: Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data 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**

Study Citation: Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)						
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age:							
Health Outcome:	Mortality	, i i un opoue, i un unun jun one pun menegen	, במו ימי				
Chemical:	•	Phthalate- Parent compound					
HERO ID:	1316219	-					
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	nce						
	Metric 1:	Test Substance Identity	High	Test substance was identified by name and CASRN.			
	Metric 2:	Test Substance Source	High	The phthalate ester was received from General Electric Company.			
	Metric 3:	Test Substance Purity	High	Purity was reported as 100% active ingredient.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Negative controls were used.			
	Metric 5:	Negative Control Response	High	Percent mortality for controls was shown in Table 3.			
	Metric 6:	Randomized Allocation	Medium	Organisms were impartially distributed into the test vessels.			
D 2. E Cl							
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	High	Static test conditions were described in detail.			
	Metric 7.	Preparation	підіі	Static test conditions were described in detail.			
	Metric 8:	Consistency of Exposure	Medium	Details of the exposure administration were reported but limited (volumes of chemicals			
	wienie o.	Administration	Mediaiii	used to make stock solutions were not reported).			
	Metric 9:	Measurement of Test Substance	High	Test concentrations were verified analytically as shown in Table 1 and described in			
		Concentration	C	Appendix I.			
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported and adequate (mortality reported at 48hr and 24hr).			
	Metric 11:	Number of Exposure Groups/	Medium	Five concentrations of DBP were tested spanning approximately one order of magnitude			
		Spacing of Exposure Levels		between the highest and lowest concentration.			
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were approximately at or below the solubility limit.			
Domain 4: Test Organis	sm						
J	Metric 13:	Test Organism Characteristics	High	Paratanytarsus parthenogenica were obtained from cultured stocks (EG&G Bionomics); age was reported as second or third instars.			
	Metric 14:	Acclimatization and Pretreatment	Medium	It was not specifically stated if organisms were acclimatized.			
	Metric 15:	Conditions Number of Organisms and	Low	Three replicate beakers per concentration with five midge larvae per beaker.			
	Metric 13.	Replicates per Group	Low	Three replicate beakers per concentration with five intuge farvae per beaker.			
Domain 5: Outcome As	esassmant		<u> </u>				
Domain 3. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described, reported and followed cited protocols.			
	Metric 17:	Outcome Assessment Methodology	Medium	There were limited details of how authors determined mortality of the test organisms.			
	Tricuic 17.			, ,			
		Cont	tinued on nex	n page			

Diisobutyl Phthalate **Environmental Hazard Evaluation** HERO ID: 1316219 Table: 1 of 1

		con	tinued from p	previous page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome:	Overall Dura Aquatic (fres	Bionomics,, EG&G (1984). Acute toxicity of twelve phthalate esters to Paratanytarsus parthenogenica (final report) report no BW-83-6-1424.  Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)  Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)  Invertebrate; Arthropods; <i>Paratanytarsus parthenogenica</i> ; Larvae				
Chemical:	•	hthalate- Parent compound				
HERO ID:	1316219	minimize Turent compound				
Domain		Metric	Rating	Comments		
	Metric 18:	Consistency of Outcome Assessment	High	Mortality assessment was conducted at 24 and 48hr and appeared to be consistent among study groups.		
Domain 6: Confoundir	ng / Variable Cor	ntrol				
	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 Preser	ntation and Analy Metric 21:	ysis Statistical Methods	High	The LC50 at 48 hr exposure was determined via moving angle analysis as described in the footnote of Table 4.		

Metric 21:	Statistical Methods	High	The LC50 at 48 hr exposure was determined via moving angle analysis as described in the footnote of Table 4.
Metric 22:	Reporting of Data	High	Outcomes were described in Table 3 and LC50 values were shown in Table 4.
Metric 23:	Explanation of Unexpected Outcomes	Medium	Outcomes were explained in the study.

Additional Comments: Authors conducted dose response of DBP and limit tests for DEHP, DIDP, and DINP. Authors report percent morality in replicate groups at 24 and 48 hr exposure. LC50 values for DBP were reported. LC50 values for DEHP, DIDP, and DINP also reported but unclear how authors obtained (or estimated) LC50 values based on a limit test.

# **Overall Quality Determination**

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
	' T ' 1 T ' 1 1 1 C ' 1 1 1 1 C   1 1 1 1 1 C   1 1 1 C   1 1 C   1 1 C   1 1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1 C   1

Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported

organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substa	ance			
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported as provided by a manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data was reported.
	Metric 3:	Test Substance Purity	High	The test substance was at least 95% pure.
Domain 2: Test Desig	n			
	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.
Domain 3: Exposure (	Characterization			
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	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 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 water solubility.
Domain 4: Test Organ	nism			
	Metric 13:	Test Organism Characteristics	Low	A source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	Appropriate acclimation for the test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.

#### Domain 5: Outcome Assessment

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 1321996 Table: 1 of 1

#### ... continued from previous page

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
	organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** 

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 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	High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confounding	g / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presen	tation and Anal	veje		
Domain 7. Data Fresch	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.
	Metric 22:	Reporting of Data	Medium	Only treatment endpoints were reported.
		1 0		, , , , , , , , , , , , , , , , , , , ,
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Animalia; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
Chemical:	Diisobutyl Phthalate- Parent compound
HERO ID:	5495608

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	The test substance was identified using electron-capture gas-liquid chromatography.
Metric 3:	Test Substance Purity	Low	The purity of the test substance was 100%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Characterizatio	n		
Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
Metric 12:		High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
Metric 15:		Low	The initial number of test organisms was not reported.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 1 of 2

#### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Animalia; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ding / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.				
Duration:	Overall Duration: 11 - 21 days; Exposure Dur				
Exposure Route,	Aquatic (marine); Water; Not determined by s	study authors (i.e., chemical of interest i	n exposure water, but unable to determine exact uptake route)		
Media, Path:					
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urch	ins, ciliates, rotifers); Animalia; Not Ap	oplicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth				
Chemical:	Diisobutyl Phthalate- Parent compound				
HERO ID:	5495608				
Domain	Metric	Rating	Comments		

Domain		Metric	Rating	Comments
Domain 1: Test Substan	nce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.
	Metric 3:	Test Substance Purity	Low	The purity of the test substance was 100%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure C	haractarization			
Domain 5. Exposure C	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-
	Wictife 7.	Preparation	Wicdiani	ported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
		Administration	8	
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose
		Spacing of Exposure Levels	C	response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	sm			
Domain 1. Test Organis	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it was assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.
		Replicates per Group		
Domain 5: Outcome As	ssessment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
		Conti	nued on next pa	ge

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 2 of 2

#### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Animalia; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confound	ding / Variable Co	ntrol		
Bomain o. Comount	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
<b>Exposure Route,</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
Chemical:	Diisobutyl Phthalate- Parent compound
HERO ID:	5495608

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	The test substance was identified using electron-capture gas-liquid chromatography.
Metric 3:	Test Substance Purity	Low	The purity of test substance was 100%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group
Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
Metric 9:	Administration Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies.
Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 1 of 2

#### ... continued from previous page

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path: Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

Metric 13:

Metric 14:

Metric 15:

Metric 16:

Metric 17:

Metric 18:

Domain 5: Outcome Assessment

**Test Organism Characteristics** 

Number of Organisms and

Adequacy of Test Conditions

Consistency of Outcome

Outcome Assessment Methodology

Replicates per Group

Conditions

Assessment

Acclimatization and Pretreatment

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine bent communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.							
Duration:				3(3):239-248.				
		Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days  Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Exposure Route, Media, Path:	Aquatic (ma	rine); water, Not determined by study author	ors (i.e., chemica	if of interest in exposure water, but unable to determine exact uptake route)				
,	T	W( A1:  - N4	l: 1	N-4 Ameliantha (a formai amalana ataulian) am N-4 Damanta d				
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Annelida</i> ; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth							
Health Outcome:								
Chemical:	•	hthalate- Parent compound						
HERO ID:	5495608							
Domain		Metric	Rating	Comments				
Domain 1: Test Substar								
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.				
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.				
Domain 2: Test Design								
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.				
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.				
Domain 3: Exposure Cl	naracterization							
- · · · · · · · · · · · · · · · · · · ·	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.				
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.				
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.				
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.				
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a do response.				
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.				

#### Continued on next page ...

Medium

Medium

Low

Low

Low

Low

population structure.

isms.

Naturally colonizing organisms were used, and it is assumed there was equal initial

All pretreatment conditions were most likely the same for control and exposed organ-

Environmental conditions were not sufficiently reported to evaluate if adequate.

Details regarding the execution of the study protocol for outcome assessment were lim-

The outcome assessment methodology (harvesting) was not clearly reported.

The initial number of test organisms was not reported.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 2 of 2

#### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Taxa, Species, Age:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Invertebrate; Worms (e.g., Annelids, Nematodes); Annelida; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confoundin	na / Variabla Ca	ntral		
Domain 6: Comoundin	U			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Preser	ntation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Arthropoda; Arthropoda; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 1: Test Substar	ice			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.
Domain 2: Test Design				
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Ch	naracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a do- response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	ım			
1000 01game	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcome As	ssessment			
		Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.

Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 1 of 2

#### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path: Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Arthropoda; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ding / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	veje		
Domain 7. Data 110s	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

HERO ID: 5495608 Table: 2 of 2

<b>Study Citation:</b>	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.					
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days					
<b>Exposure Route,</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Arthropoda; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Development/Growth					
Chemical:	Diisobutyl Phthalate- Parent compound					
HERO ID:	5495608	•				
Domain		Metric	Rating	Comments		
Domain 1: Test Substan	ce					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.		
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.		
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.		
Domain 2: Test Design						
Č	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		

	Metric 2: Metric 3:	Test Substance Source Test Substance Purity	High Low	The test substance was identified using electron-capture gas-liquid chromatography. Purity of the test substance was 100%.
Domain 2: Test De	esign			
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposu	re Characterization			
1	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/ 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 Or	rganism			
Boniani i. Test of	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcom	ne 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 (harvesting) 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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Diisobutyl Phthalate Environmental Hazard Evaluation HERO ID: 5495608 Table: 2 of 2

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Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Arthropods; Arthropoda; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confoundin	g / Variable Co	ntrol		
Domain o. Comounam	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presen	tation and Anal	vsis		
Domain 7. Data 1 resen	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

<b>Study Citation:</b>	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.							
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days							
<b>Exposure Route,</b>	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)							
Media, Path:	•	•	•					
Taxa, Species, Age:	: Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Chordata</i> ; multiple species; Not Applicable (e.g., fungi or algae studies							
Health Outcome: Chemical: HERO ID:	Reported Developmen Diisobutyl I 5495608	nt/Growth Phthalate- Parent compound						
Domain		Metric	Rating	Comments				
Domain 1: Test Substan	ce							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.				
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.				
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.				
Domain 2: Test Design								
_	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.				
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.				
	Matria 6.	Dandamizad Allagation	Low	December 414 act and the initial all accounts for a surface and determined				

		<b>-</b>		•		
Domain 2: Test Design						
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.		
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.		
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.		
Domain 3: Exposure Cl						
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.		
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.		
	M-4-:- 0.	Administration Measurement of Test Substance	TT: _1.			
	Metric 9:	Concentration	High	Exposure concentrations were measured using appropriate analytical technologies.		
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.		
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose		
		Spacing of Exposure Levels		response.		
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.		
Domain 4: Test Organis						
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.		
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-		
		Conditions		isms.		
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.		
		Replicates per Group				
Domain 5: Outcome As	ssessment					
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.		
	Continued on next page					
		Cont		·····		

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Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Chordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not

Reported

Health Outcome: Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Prese	entation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	-	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic							
Duration:	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.  Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days								
Exposure Route,		, I	•	al of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:	riquatio (iii	ame), water, rist determined by study	additions (new, entermine	ar of interest in outpostate water, our annote to determine office apartic route,					
Taxa, Species, Age:	Invertebrate	: Other Invertebrate (e.g., sea urchins	. ciliates, rotifers): C	Chordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not					
, ~ <b>F</b> , <b>g</b>	Reported	,	,,,,, -	······································					
Health Outcome:	Development/Growth								
Chemical:	Diisobutyl I	Phthalate- Parent compound							
HERO ID:	5495608	•							
Domain		Metric	Rating	Comments					
Domain 1: Test Substar	nce								
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.					
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.					
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.					
Domain 2: Test Design									
Z.	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.					
				assessed outcomes.					

	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.
Domain 2: Test Design				
Domain 2. Test Design	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure C	h ama atami mati an			
Domain 3. Exposure Ci		E ' 110 / /E /M !'	3.4.1	
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Concentration Exposure Duration and Frequency	Uiah	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/	High High	The number of exposure groups and spacing of exposure levels were suitable for a dose
	Meure 11.	Spacing of Exposure Levels	nigii	response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	zm			
Bolliam 1. Test Organis	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-
		Conditions		isms.
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.
		Replicates per Group		
Domain 5: Outcome As	ssessment			
2 cmain 5. Gateome 7 h	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
		Conti	nued on next pa	age

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Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic **Study Citation:** 

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Chordata; multiple species; Not Applicable (e.g., fungi or algae studies) or Not

Reported

**Health Outcome:** Development/Growth

**Chemical:** Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confounding	g / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Present	ation and Anal	veic		
Domain 7. Data 1 resent	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

Study Citation: Duration: Exposure Route, Media, Path:	ge: Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Coelenterata</i> ; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:							
Domain		Metric	Rating	Comments			
Domain 1: Test Substand							
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
Ü	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.			
Domain 3: Exposure Ch	aracterization Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test modic proporation mothods your adequately re-			
		Experimental System/Test Media Preparation		The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.			
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organisi	m						
Commin 1. 10st Organisi	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.			
Domain 5: Outcome Ass	sessment						
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
		Conti	nued on next pa	nge			

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Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Coelenterata; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic						
Duration:		s exposed to di-normal-butyl phthalate. Aquation: 11 - 21 days; Exposure Duration: 11		3(3):239-248.			
Exposure Route, Media, Path:	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)  Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Coelenterata</i> ; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Taxa, Species, Age:							
Health Outcome: Chemical:	Developmen						
HERO ID:	Diisobutyl Phthalate- Parent compound 5495608						
Domain		Metric	Rating	Comments			
Domain 1: Test Substanc		T (0.1)					
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2: Metric 3:	Test Substance Source Test Substance Purity	High Low	The test substance was identified using electron-capture gas-liquid chromatography.  Purity of the test substance was 100%.			
	Meure 3.	rest Substance Furity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.			
Domain 3: Exposure Cha	aracterization						
Domain 3. Exposure Cir	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-			
		Preparation		ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose			
		Spacing of Exposure Levels		response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organisr	n						
C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and	Low	The initial number of test organisms was not reported.			
		Replicates per Group					
Domain 5: Outcome Ass	sessment						
Zomani J. Gutcome 1100	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.			
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.			
			nued on next pa				

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Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Coelenterata; Actinaria; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confoundi	na / Variabla Ca	ntral		
Domain 6. Comound	C			
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Prese	ntation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

<b>Study Citation:</b>	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic							
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.							
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days						
Exposure Route,	Aquatic (marine); Water; Not determined by stud	ly authors (i.e., chemical of interest	in exposure water, but unable to determine exact uptake route)					
Media, Path:								
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Echinodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported							
<b>Health Outcome:</b>	Development/Growth							
Chemical:	Diisobutyl Phthalate- Parent compound							
HERO ID:	5495608							
Domain	Metric	Rating	Comments					

Domain		Metric	Rating	Comments
Domain 1: Test Substance	e			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.
Domain 2: Test Design				
Č	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Cha	aracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism	n			
Domain I. Test ergamsi.	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcome Ass	essment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
		Conti	inued on next pa	ge

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Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Echinodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Co	ntrol		
	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions
		Design and Procedures		
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	lysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.						
Duration: Exposure Route, Media, Path:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days  Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route						
Taxa, Species, Age: Health Outcome:	Developmen	nt/Growth	es, rotifers); Echi	nodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Chemical: HERO ID:	Diisobutyl Phthalate- Parent compound 5495608						
Domain		Metric	Rating	Comments			
Domain 1: Test Substan	ce		-				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.			
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.			
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.			
Domain 2: Test Design							
C	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.			
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.			
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.			
Damain 2, Evmagum Ch	ana atanizatian						
Domain 3: Exposure Ch	Metric 7:	Experimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-			
	medie 7.	Preparation	Mediani	ported but there is some concern over the use of plastic trays.			
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.			
		Administration					
	Metric 9:	Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.			
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.			
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose			
		Spacing of Exposure Levels		response.			
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.			
Domain 4: Test Organis	m						
C	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.			
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.			
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.			
D : 5 O							
Domain 5: Outcome As		Adams of Test C	T	Emilian and a delica and a second of the sec			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.			
	Metric 17: Metric 18:	Outcome Assessment Methodology Consistency of Outcome Assessment	Low Low	The outcome assessment methodology (harvesting) was not clearly reported.  Details regarding the execution of the study protocol for outcome assessment were limited.			

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Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Echinodermata; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confound	ding / Variable Co	ntrol		
Bomain o. Comount	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Design and Procedures Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

<b>Study Citation:</b>	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Mollusks; Mollusca; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Metric 1: Te Metric 2: Te Metric 3: Te	Metric  est Substance Identity est Substance Source est Substance Purity  egative Controls egative Control Response	Rating  Low High Low  High	Comments  The chemical was identified by name only.  The test substance was identified using electron-capture gas-liquid chromatography.  Purity of the test substance was 100%.
Metric 2: Te Metric 3: Te	est Substance Source est Substance Purity egative Controls	High Low	The test substance was identified using electron-capture gas-liquid chromatography.
Metric 2: Te Metric 3: Te	est Substance Source est Substance Purity egative Controls	High Low	The test substance was identified using electron-capture gas-liquid chromatography.
Metric 3: Te	est Substance Purity egative Controls	Low	
	egative Controls		Purity of the test substance was 100%.
	2	High	
Domain 2: Test Design	2	High	
2	egative Control Response		Study authors reported using a concurrent negative control group.
Metric 5: No		High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
Metric 6: Ra	andomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Characterization			
*	xperimental System/Test Media	Medium	The experimental system and/or test media preparation methods were adequately re-
	reparation	Wedium	ported but there is some concern over the use of plastic trays.
	onsistency of Exposure	High	Exposures were administered consistently across study groups.
Ac	dministration	8	,,,,,,,, .
	leasurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	oncentration xposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	umber of Exposure Groups/	•	
	pacing of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a dose response.
	esting at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
3.3333 327		8	
Domain 4: Test Organism			
Metric 13: Te	est Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
Metric 14: Ac	cclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organ-
	onditions		isms.
Metric 15: No	umber of Organisms and	Low	The initial number of test organisms was not reported.
Re	eplicates per Group		
Domain 5: Outcome Assessment			
Metric 16: Ac	dequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Conti	nued on next pa	ge

### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route,

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Mollusks; Mollusca; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ding / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	sentation and Anal	veje		
Domain 7. Data 110	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983).	Alterations in composition of field-developed and laboratory-developed estuarine benthic
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communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Invertebrate; Mollusks; Molluska; multiple species; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** 

Development/Growth

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 1: Test Substan				
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable for assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure Ch	aracterization			
r	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Concentration Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/	High	The number of exposure groups and spacing of exposure levels were suitable for a do
		Spacing of Exposure Levels		response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organis	m			
Zomani ii 10st Organis	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcome As	sessment			
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if adequate.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lin
	1.100.10	Assessment	20,,	ited.
		Conti	nued on next pa	ong a

### ... continued from previous page

Study Citation: Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic

communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path:

Taxa, Species, Age: Invertebrate; Mollusks; Mo

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
Domain 6: Confound	ling / Variable Co	ntrol		
Domain o. Comouna	Metric 19:	Confounding Variables in Test	High	There were no reported differences among the study groups in environmental conditions.
		Design and Procedures	C	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Dania 7, Data Bara				
Domain 7: Data Prese	entation and Anai	ysis		
	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	<b>Explanation of Unexpected Outcomes</b>	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

### **Overall Quality Determination**

Study Citation:	Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic
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organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path:

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age:

Invertebrate; Arthropods; Mysidopsis bahia; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
Domain 1: Test Substar	ice			
	Metric 1:	Test Substance Identity	Low	The test substance nomenclature was reported without a CASRN.
	Metric 2:	Test Substance Source	Low	The source was reported as provided by manufacturer from commercially available batches. Manufacture name and batch number were not provided. No analytical data
	Metric 3:	Test Substance Purity	High	was reported.  The test substance was at least 95% pure.
Domain 2: Test Design				
20114111 21 1000 2001811	Metric 4:	Negative Controls	High	A negative control was reported.
	Metric 5:	Negative Control Response	High	The control response was acceptable.
	Metric 6:	Randomized Allocation	Low	An allocation method was not reported.
Domain 3: Exposure Cl	naracterization			
r	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system was well described. However, headspace or measures to prevent volatilization were not reported.
	Metric 8:	Consistency of Exposure Administration	High	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 end of the test. In static studies, final test concentrations frequently were 50% of the initial concentrations. Loss of the phthalate esters was thought to be principally due to adsorption to the test vessels.
	Metric 10:	Exposure Duration and Frequency	High	Duration and frequency of exposure were appropriate for the test.
	Metric 11:	Number of Exposure Groups/ 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 water solubility.
Domain 4: Test Organis	sm			
C	Metric 13:	Test Organism Characteristics	Low	A source was not reported.
	Metric 14:	Acclimatization and Pretreatment	High	An appropriate acclimation for the test was reported.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Medium	Each test was performed using duplicate test concentrations with 10 organisms per test vessel.

#### Domain 5: Outcome Assessment

#### ... continued from previous page

Study Citation: Adams, W. J., Biddinger, G. R., Robillard, K. A., Gorsuch, J. W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9):1569-1574.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Exposure Route,** 

Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Media, Path: Taxa, Species, Age:

Chemical:

Invertebrate; Arthropods; Mysidopsis bahia; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome:

Mortality

Diisobutyl Phthalate- Parent compound

**HERO ID:** 1321996; Linked HERO ID(s): 1321996, 1316224

Domain		Metric	Rating	Comments
I	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were appropriate for the test.
I	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
I	Metric 18:	Consistency of Outcome	High	Outcome assessment was consistent across groups.
		Assessment		
Domain 6: Confounding /	Variable Con	trol		
I	Metric 19:	Confounding Variables in Test	High	Environmental conditions were consistant across groups.
		Design and Procedures		
1	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences between groups.
Domain 7: Data Presentation and Analysis				
I	Metric 21:	Statistical Methods	High	Statistical methods were performed and described.
I	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>	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Rhynchocoela; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
Chemical:	Diisobutyl Phthalate- Parent compound
HERO ID:	5495608

Domain		Metric	Rating	Comments
Domain 1: Test Substa	nce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	High	The test substance was identified using electron-capture gas-liquid chromatography.
	Metric 3:	Test Substance Purity	Low	Purity of the test substance was 100%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using a concurrent negative control group.
	Metric 5:	Negative Control Response	High	The biological response of the negative control group was reported and reasonable fo assessed outcomes.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how initial placement of aquaria was determined.
Domain 3: Exposure C	haracterization			
	Metric 7:	Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but there is some concern over the use of plastic trays.
	Metric 8:	Consistency of Exposure	High	Exposures were administered consistently across study groups.
	Metric 9:	Administration Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure was suitable for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were suitable for a do response.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organi	sm			
	Metric 13:	Test Organism Characteristics	Medium	Naturally colonizing organisms were used, and it is assumed there was equal initial population structure.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	All pretreatment conditions were most likely the same for control and exposed organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The initial number of test organisms was not reported.
Domain 5: Outcome A	ssessment			
				Environmental conditions were not sufficiently reported to evaluate if adequate.

### ... continued from previous page

Study Citation:	Tagatz, M. E., Deans, C. H., Moore, J. C., Plaia, G. R. (1983). Alterations in composition of field-developed and laboratory-developed estuarine benthic
	communities exposed to di-normal-butyl phthalate. Aquatic Toxicology 3(3):239-248.

**Duration:** Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days

Exposure Route, Media, Path: Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Taxa, Species, Age: Health Outcome:

Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); Rhynchocoela; Not Applicable (e.g., fungi or algae studies) or Not Reported

**Health Outcome:** Development/Growth

Chemical: Diisobutyl Phthalate- Parent compound

**HERO ID:** 5495608

Domain		Metric	Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology (harvesting) was not clearly reported.
	Metric 18:	Consistency of Outcome	Low	Details regarding the execution of the study protocol for outcome assessment were lim-
		Assessment		ited.
Domain 6: Confound	ling / Variable Cor	ntrol		
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Pres	entation and Anal	veic		
Domain 7. Data 110s	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: None

# **Overall Quality Determination**

HEBU	ID.	51037	Table:	1 of
	' I I <i>I</i>	1191/	Table	1 ()1

<b>Study Citation:</b>	Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979)	. The acute toxicity of 78 chemicals and pestici	de formulations against two brackish
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water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

**Duration:** 

Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) **Exposure Route,** Media, Path:

Taxa, Species, Age: Invertebrate; Arthropods; Nitocra spinipes; Adult

**Health Outcome:** Mortality

Diisobutyl Phthalate- Parent compound Chemical:

**HERO ID:** 51937

Domain		Metric	Rating	Comments
Domain 1: Test Substar	nce			
	Metric 1:	Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2:	Test Substance Source	Low	The source was not reported.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of the test substance were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure C	haracterization			
2 c 0. 2pccu.c C	Metric 7:	Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations. They stated that "No control analyses for the actual substance(s) were made of the test solutions."
	Metric 8:	Consistency of Exposure Administration	Medium	Only general methods of exposure administration were reported so assessment was difficult to determine.
	Metric 9:	Measurement of Test Substance	Low	Exposure concentrations were not measured.
	Metric 10:	Concentration Exposure Duration and Frequency	Medium	The duration of exposure and exposure frequency were reported and suitable, but slightly longer than typical for the study type (96h).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	At least six concentrations were tested, but the range of exposure groups was not reported.
	Metric 12:	Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit. However, the reported LC50, 1.7 mg/L, is below the solubility reported in the Final Scope for DBP (11.2 mg/L at 25C).
Domain 4: Test Organia	sm			
	Metric 13:	Test Organism Characteristics	Medium	The source of the test animals was not reported.
	Metric 14:	Acclimatization and Pretreatment	Low	It is unclear if test organisms were acclimatized to test conditions.
	Metric 15:	Conditions Number of Organisms and Replicates per Group	Low	Twenty organisms with no replicates per treatment were used.

#### Domain 5: Outcome Assessment

### ... continued from previous page

Study Citation: Linden, E., Bengtsson, B. E., Svanberg, O., Sundstrom, G. (1979). The acute toxicity of 78 chemicals and pesticide formulations against two brackish

water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitocra spinipes. Chemosphere 8(11-12):843-851.

**Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)

Exposure Route, Media, Path: Aquatic (brackish); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)

Toyo Species A

**Taxa, Species, Age:** Invertebrate; Arthropods; *Nitocra spinipes*; Adult

**Health Outcome:** Mo

Mortality

Chemical:

Diisobutyl Phthalate- Parent compound

**HERO ID:** 51937

Domain		Metric	Rating	Comments
N.	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions of the test system were conducive to maintenance of organism health.
M	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 / V	/ariable Coi	ntrol		
N.	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
N	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentatio	on and Anal	ysis		
M	Metric 21:	Statistical Methods	High	Statistical methods were adequately described.
N.	Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group, but results were described in Table 3.
$\mathbf{N}$	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

# **Overall Quality Determination**