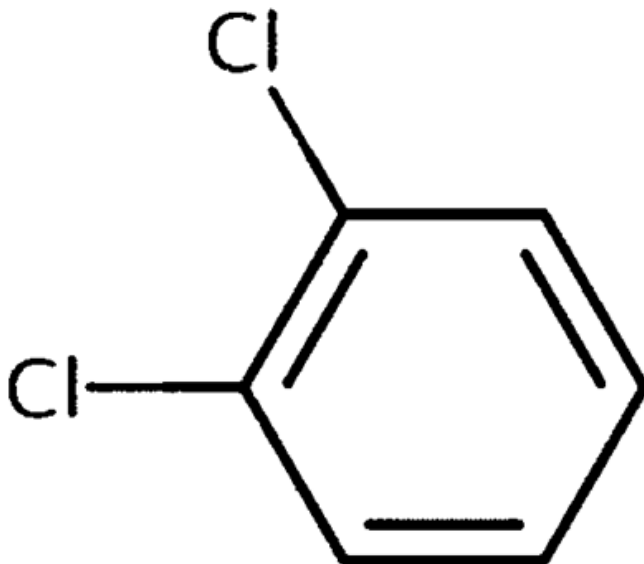


**Draft Data Quality Evaluation Information for
Environmental Hazard for
o-Dichlorobenzene**

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 95-50-1



April 2026

This supplemental file contains information regarding the data quality evaluation results relevant to the characterization of environmental hazard for the *Draft Human Health and Environmental Hazard Assessment for o-Dichlorobenzene*. 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 will be described in the *Draft Systematic Review Protocol for o-Dichlorobenzene*.

Different data quality evaluation forms were used for different organism as described in the PECO statement in Appendix H.5.12 of the 2021 Draft Systematic Review Protocol. Each health outcome was evaluated independently within a given reference; therefore, each reference may have more than one overall quality determination (OQD) to more appropriately reflect the quality of each health outcome and the respective hazard endpoints as described by the study authors. Some data evaluation forms have general additional comments presented adjacent to the OQD to add further context. No OQD is determined for each reference as a whole, if it contains data from more than one evidence stream. The study details and respective endpoints are organized by first the relevant habitat (i.e., aquatic, terrestrial), then taxa categories (e.g., vertebrates, invertebrates, vegetation) followed by taxonomic groups (e.g., fish, amphibian, mammalian, avian, worms, vascular plants), individual species, and finally exposure duration and health outcome (e.g., mortality) categories relevant to the endpoint being evaluated.

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HERO ID	Reference	Page
<i>o</i>-Dichlorobenzene		
Habitat: Aquatic (freshwater)		
Taxa: Vertebrates		
<i>Brachydanio rerio</i>		
4930936	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.	11
<i>Carassias auratus</i>		
654548	Liu, Z. T., Wang, L. S., Chen, S. P., Li, W., Yu, H. X. (1996). Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. <i>Bulletin of Environmental Contamination and Toxicology</i> 57(3):421-425.	14
<i>Catla catla L.</i>		
3345050	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla L.</i> using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.	18
3345050	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla L.</i> using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.	20
<i>Cyprinus carpio</i>		
647011	Zhao, Y. H., Wang, L. S., Gao, H., Zhang, Z. (1993). Quantitative structure–activity relationships: Relationship between toxicity of organic chemicals to fish and to photobacterium phosphoreum. <i>Chemosphere</i> 26(11):1971-1979.	24
<i>Danio rerio</i>		
1417745	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. <i>Environmental Toxicology and Chemistry</i> 22(2):329-335.	26
1417745	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. <i>Environmental Toxicology and Chemistry</i> 22(2):329-335.	28
<i>Lepomis macrochirus</i>		
18064	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (<i>Lepomis macrochirus</i>). <i>Bulletin of Environmental Contamination and Toxicology</i> 26(4):446-452.	38
18050	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (<i>Lepomis macrochirus</i>). :379-392.	40
<i>Oncorhynchus mykiss</i>		

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7494661	Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Morairity, A., Richter, J. (1984). Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – parts I and II.	43
	<i>Pimephales promelas</i>	
1392282	Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. Water Research 13(2):137-141.	47
32170	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.	49
654569	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.	64
5253177	Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. Ecotoxicology and Environmental Safety 35(3):236.	69
11455129	Hall, L., Kier, L., Phipps, G. (1984). STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON THE TOXICITIES OF BENZENE-DERIVATIVES .1. AN ADDITIVITY MODEL. Environmental Toxicology and Chemistry 3(3):355-365.	75
	<i>Poecilia reticulata</i>	
654569	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.	78
	<i>Rana pipiens</i>	
93660	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.	82
	<i>Salmo gairdneri</i>	
4930936	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12(2):253-262.	84
3634370	Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.	87
93660	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.	89
	<i>Salmo gairdneria</i>	
2048390	Dow Chemical, (1974). Dynamic acute toxicity of selected phenol and benzene compounds to rainbow trout, <i>Salmo gairdneria</i> , Richardson prepared by Waste Control Dept.	91

Taxa: Invertebrates*Ceriodaphnia dubia*

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651551	Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran <i>Ceriodaphnia cf. dubia</i> . <i>Archives of Environmental Contamination and Toxicology</i> 34(3):248-252.	95
	<i>Chironomus dilutus</i>	
11799635	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).	97
12834309	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.	105
	<i>Daphnia magna</i>	
1486051	Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. <i>Aquatic Toxicology</i> 8(3):163-174.	114
4930936	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.	116
660799	Deneer, J. W., Sinnige, T. L., Seinen, W., Hermens, J. L. (1988). The joint acute toxicity to <i>Daphnia magna</i> of industrial organic chemicals at low concentrations. <i>Aquatic Toxicology</i> 12(1):33-38.	119
773229	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.	121
2777011	Pawlisz, A. V., Peters, R. H. (1995). Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 29(3):613-621.	123
2779031	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2795-2800.	125
5255675	Pawlisz, A. V., Peters, R. H. (1993). A test of the equipotency of internal burdens of nine narcotic chemicals using <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2801-2806.	134
651551	Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran <i>Ceriodaphnia cf. dubia</i> . <i>Archives of Environmental Contamination and Toxicology</i> 34(3):248-252.	137
4930936	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.	139
773229	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.	141
	<i>Tanytarsus dissimilis</i>	
3634370	Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.	145

Taxa: Plants (Non-vascular)*Chlorella pyrenoidosa*

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3607057	Zhang, S., Lin, D., Wu, F. (2016). The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae. <i>Journal of Hazardous Materials</i> 311:186-193.	147
	<i>Chlorella vulgaris</i>	
5162728	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.	151
	<i>Cyclotella meneghiniana</i>	
659965	Figueroa, I. D., Simmons, M. S. (1991). Structure-activity relationships of chlorobenzenes using DNA measurement as a toxicity parameter in algae. <i>Environmental Toxicology and Chemistry</i> 10(3):323-330.	157
	<i>Scenedesmus subspicatus</i>	
1023091	Kühn, R., Pattard, M. (1990). Results of the harmful effects of water pollutants to green algae (<i>Scenedesmus subspicatus</i>) in the cell multiplication inhibition test. <i>Water Research</i> 24(1):31-38.	159
5162728	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.	161
	<i>Selenastrum capricornutum</i>	
1990600	Galassi, S., Vighi, M. (1981). Testing toxicity of volatile substances with algae. <i>Chemosphere</i> 10(10):1123-1126.	167
	<i>Selenastrum capricornutum</i>	
4930936	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.	169
1333375	Casserly, D. M., Davis, E. M., Downs, T. D., Guthrie, R. K. (1983). Sorption of organics by <i>Selenastrum capricornutum</i> . <i>Water Research</i> 17(11):1591-1594.	173
5162728	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.	177
Taxa: Plants (Vascular)		
	<i>Bacopa caroliniana,</i>	
1998227	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.	183
	<i>Hydrilla verticillata</i>	
1998227	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.	185
	<i>Myriophyllum spicatum</i>	

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1998227	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.	187
Taxa: Other		
	<i>Tetrahymena pyriformis</i>	
676758	Yoshioka, Y., Ose, Y., Sato, T. (1985). Testing for the toxicity of chemicals with <i>Tetrahymena pyriformis</i> . <i>Science of the Total Environment</i> 43(1-2):149-157.	189
Habitat: Aquatic (marine)		
Taxa: Vertebrates		
	<i>Cyprinodon variegatus</i>	
18110	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows (<i>Cyprinodon variegatus</i>). <i>Bulletin of Environmental Contamination and Toxicology</i> 27(5):596-604.	191
	<i>Solea solea</i>	
5253054	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). <i>Bulletin of Environmental Contamination and Toxicology</i> 54(1):36-42.	193
Taxa: Invertebrates		
	<i>Artemia</i>	
1486051	Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. <i>Aquatic Toxicology</i> 8(3):163-174.	195
	<i>Melita longidactyla</i>	
1002318	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . <i>Chemosphere</i> 80(1):20-27.	197
1002318	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . <i>Chemosphere</i> 80(1):20-27.	199
	<i>Palaemon pugio</i>	
5352394	Curtis, M., Ward, C. (1981). Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. <i>Journal of Hydrology</i> 51(1):359-367.	205
	<i>Palaemonetes pugio</i>	
1392282	Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. <i>Water Research</i> 13(2):137-141.	208
	<i>Paracentrotus lividus</i>	

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1003942	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.	210
	<i>Strongylocentrotus purpuratus</i>	
5255526	Oshida, P. S. (1977). Toxicity of a chlorinated benzene to sea urchin embryos.	218
Taxa: Plants (Non-vascular)		
	<i>Chlorella marine</i>	
5243980	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. <i>Chinese Journal of Oceanology and Limnology</i> 15(4):308-313.	220
1982535	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.	222
5257521	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.	224
	<i>Nannochloris oculata</i>	
5257521	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.	228
	<i>Nannochloropsis oculata</i>	
5243980	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. <i>Chinese Journal of Oceanology and Limnology</i> 15(4):308-313.	232
1982535	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.	234
	<i>Phaeodactylum tricorutum</i>	
5243980	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. <i>Chinese Journal of Oceanology and Limnology</i> 15(4):308-313.	236
1982535	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.	238
5257521	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.	240
	<i>Platymonas subcordiformis</i>	
5243980	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. <i>Chinese Journal of Oceanology and Limnology</i> 15(4):308-313.	244

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1982535	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.	246
5257521	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.	248
	<i>Pyramidomonas sp.</i>	
5243980	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. <i>Chinese Journal of Oceanology and Limnology</i> 15(4):308-313.	252
1982535	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.	254
	<i>Pyramimonas sp.</i>	
5257521	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.	256

Habitat: Aquatic (brackish)**Taxa: Vertebrates***Platichthys flesus*

5253054	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). <i>Bulletin of Environmental Contamination and Toxicology</i> 54(1):36-42.	260
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Taxa: Invertebrates*Acartia bifilosa*

5246949	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.	262
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Acartia clausi

5246949	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.	265
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Eurytemora affinis

5246949	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.	268
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Habitat: Terrestrial**Taxa: Invertebrates**

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	<i>Eisenia fetida</i>		
3625226		Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm <i>Eisenia fetida</i> . <i>Journal of Environmental Quality</i> 14(3):383-388.	272
Taxa: Plants (Vascular)			
	<i>Glycine max</i>		
5265635		Kraaij, H., Connell, D. W. (1997). Bioconcentrations and uptake kinetics of chlorobenzenes in soy-bean roots. <i>Chemosphere</i> 34(12):2607-2620.	274
	<i>Holcus lanatus</i>		
1003825		Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. <i>Chemosphere</i> 53(5):583-591.	276
1003825		Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. <i>Chemosphere</i> 53(5):583-591.	278
Taxa: Other			
	<i>Kingdom Fungi</i>		
2725058		Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. <i>Environmental Toxicology and Chemistry</i> 17(8):1462-1468.	280
2725058		Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. <i>Environmental Toxicology and Chemistry</i> 17(8):1462-1468.	282
	<i>Name not given</i>		
5256918		Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. <i>Environmental Toxicology and Chemistry</i> 18(9):1891-1898.	284
5256918		Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. <i>Environmental Toxicology and Chemistry</i> 18(9):1891-1898.	286
	<i>No name given</i>		
5262745		Liang, H., Chen, A., Ding, C., Li, Z. (2011). Influence of 1,2-Dichlorobenzene on Microbial and Enzyme Activities in Wetland Soil. <i>Procedia Environmental Sciences</i> 10:122-127.	288

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.			
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; <i>Brachydanio rerio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.	
	Metric 3: Test Substance Purity	Low	The percent purity was not reported. All test chemicals were reported to be of analytical grade.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Limited details were given in the paper with respect to a control. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group for the test substance was not reported.	
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study reported the use of two closed glass bottles for each concentration, but major details regarding test media preparation were not given in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration details are not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured at the beginning and the end of the short-term tests, but they were not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 10: Exposure Duration and Frequency	Low	This was an acute toxicity test conducted for 48 h. According to OECD guidelines for Test No. 203, acute fish toxicity tests should be conducted for 96 h.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups and spacing of exposure levels in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 12: Testing at or Below Solubility Limit	Low	No details were provided to determine whether exposure concentrations exceeded the water solubility limit.	

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Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.
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; <i>Brachydanio rerio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the organisms was not reported, but the test organism was appropriate for evaluating the outcome. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 5 fish per concentration and 2 replicates for each concentration. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions during the test were reported, but it could not be determined whether differences occurred between control and exposed populations. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 18:	Consistency of Outcome Assessment	Low	No details were provided regarding the execution of the study protocol for outcome assessment.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information presented to suggest differences among treatment groups were due to exposure unrelated reasons.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described well.
Metric 22:	Reporting of Data	Low	Mortality data for treatments groups and controls were not reported. Only 48h LC 50 values were reported.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

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Study Citation: Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12(2):253-262.
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; *Brachydanio rerio*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Additional Comments:	The source of test organisms, exposure concentrations (nominal or measured), spacing of exposure levels, and control response were not reported. Only 48 h LC 50 values along with confidence intervals were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Liu, Z. T., Wang, L. S., Chen, S. P., Li, W., Yu, H. X. (1996). Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. <i>Bulletin of Environmental Contamination and Toxicology</i> 57(3):421-425.			
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; <i>Carassias auratus</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	654548			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.	
	Metric 2: Test Substance Source	Low	The chemical source was reported, but not any analytical verification.	
	Metric 3: Test Substance Purity	High	The purity was reported as 95% or greater.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Use of a control is implied with calculating the LC50. They also cited OECD methods but made no mention of using a control.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Some details were provided on the test system used. No details were provided on preparation of the test substance.	
	Metric 8: Consistency of Exposure Administration	Medium	Downgraded due to minimal information on exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	The duration was shorter than usual for a fish acute test (48 hours).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups was reported as four to six, but no actual test concentration values were given. An LC50 value was determined.	
	Metric 12: Testing at or Below Solubility Limit	Medium	LC50 value was well below the limit of solubility, but the range of test concentrations used was not reported.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There is slight uncertainty of the age of fish used. Each fish was approximately 3.5g and 4.0cm in length.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	A ten-day acclimation period was reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were four organisms per test container and two replicates per test concentration.	
Domain 5: Outcome Assessment				

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Study Citation: Liu, Z. T., Wang, L. S., Chen, S. P., Li, W., Yu, H. X. (1996). Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. *Bulletin of Environmental Contamination and Toxicology* 57(3):421-425.

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; *Carassias auratus*; Juvenile

Health Outcome: Mortality

Chemical: o-Dichlorobenzene

HERO ID: 654548

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate and clearly reported. They followed OECD guidelines for testing.
	Metric 17: Outcome Assessment Methodology	Medium	Methodology was not thoroughly reported.
	Metric 18: Consistency of Outcome Assessment	Medium	Since minimal information was provided on outcome methodology, it is difficult to determine if inconsistencies were present.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no confounding variables in the test design or procedures. Fish were acclimated to test conditions and environmental conditions during the test were noted.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences between test organisms that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Authors used probit transformation in determining LC50s.
	Metric 22: Reporting of Data	Low	Only LC50 values were reported. No raw data was shown.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Liu, Z. T., Wang, L. S., Chen, S. P., Li, W., Yu, H. X. (1996). Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. <i>Bulletin of Environmental Contamination and Toxicology</i> 57(3):421-425.			
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; <i>Carassias auratus</i> ; Juvenile			
Health Outcome:	Mechanistic-Liver toxicology			
Chemical:	o-Dichlorobenzene			
HERO ID:	654548			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.	
	Metric 2: Test Substance Source	Low	The chemical source was reported, but not any analytical verification.	
	Metric 3: Test Substance Purity	High	The purity was reported as 95% or greater.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	The use of a control was not reported.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Some details were provided on the test system used. No details were provided on preparation of the test substance.	
	Metric 8: Consistency of Exposure Administration	Medium	Downgraded due to minimal information on exposure administration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	Medium	Fish were only exposed for 24 hours before liver tissue was assessed for ATP content.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	The number of exposure groups was reported as four to six, but no actual test concentration values were given.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The range of test concentrations used was not reported. The LC50 value reported was below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	There is slight uncertainty of the age of fish used. Each fish was approximately 3.5g and 4.0cm in length.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	A ten-day acclimation period was reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were four organisms per test container and two replicates per test concentration.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate and clearly reported. They followed OECD guidelines for testing.	
	Metric 17: Outcome Assessment Methodology	Medium	The procedure to assess ATP content in the liver tissue was described. It is unclear how many fish livers per test concentration were analyzed for ATP content.	

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Study Citation:	Liu, Z. T., Wang, L. S., Chen, S. P., Li, W., Yu, H. X. (1996). Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. <i>Bulletin of Environmental Contamination and Toxicology</i> 57(3):421-425.
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; <i>Carassias auratus</i> ; Juvenile
Health Outcome:	Mechanistic-Liver toxicology
Chemical:	o-Dichlorobenzene
HERO ID:	654548

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Medium	Since some information is lacking, it is hard to know if some inconsistencies were present.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no confounding variables in the test design or procedures. Fish were acclimated to test conditions and environmental conditions during the test were noted.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information provided to suggest differences between test organisms that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Some information was given on calculations involving the ATP analysis.
	Metric 22: Reporting of Data	Medium	The ATP value was given for the chemical in Table 1. Raw data was not shown (if more than one fish liver tissue per test concentration was used).
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: None

Overall Quality Determination**Medium**

Study Citation:	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla</i> L. using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.
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; <i>Catla catla</i> L.; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	3345050; Linked HERO ID(s): 3345050, 5253108

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
Metric 2:	Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	The percent purity was reported as 99%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	An appropriate solvent control was reported.
Metric 5:	Negative Control Response	Low	No control response was reported in the acute test.
Metric 6:	Randomized Allocation	Low	Researchers did not report how organisms were 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.
Metric 8:	Consistency of Exposure Administration	Low	Details of the exposure administration were not reported adequately to determine if they were consistent.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured in the acute study.
Metric 10:	Exposure Duration and Frequency	High	A standard acute duration was used for this 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. They were adequate to address the purpose of the study.
Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the test concentrations were well under the solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	There are uncertainties about the characteristics of test organisms. The test organisms were reported to be from a local farm, but the name was not provided. The age of the organisms was also not reported, but the lengths and the weights were reported.
Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for 10 days prior to the study.
Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and the number of replicates were not reported.
Domain 5: Outcome Assessment			

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Study Citation:	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla</i> L. using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.
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; <i>Catla catla</i> L.; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	3345050; Linked HERO ID(s): 3345050, 5253108

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology reported the intended outcome with few details.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The test organisms were reported to be acclimated, but the test conditions were not reported in great detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis for the acute exposure was described adequately. Probit graph analysis method was employed to calculate the LC50 value.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. Only an LC50 value was reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability. 95% confidence intervals were not reported.

Additional Comments: This evaluation was used for the acute exposure of *Catla catla* to 1,2-DCB. A 96h LC50 value was reported as 1.4mg/L.

Overall Quality Determination

Low

Study Citation:	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla</i> L. using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.			
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; <i>Catla catla</i> L.; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	3345050; Linked HERO ID(s): 3345050, 5253108			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test 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 percent purity was reported as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	An appropriate solvent control was reported.	
	Metric 5: Negative Control Response	High	No mortality was reported, and the control response to DNA damage was reported and appropriate.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure.	
	Metric 8: Consistency of Exposure Administration	Low	Details of exposure administration were not reported, making it difficult to assess consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were only measured in the highest concentration/stock solution.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response by study authors. They were adequate to address the purpose of the study.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the exposure levels were well below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from a local farm, but an actual name was not reported. The age of the fish was also not reported, but the lengths and the weights were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for 10 days prior to the start of the study.	
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Study Citation: Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, *Catla catla* L. using alkaline comet assay. *Bulletin of Environmental Contamination and Toxicology* 91(6):616-622.

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; *Catla catla* L.; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mortality

Chemical: o-Dichlorobenzene

HERO ID: 3345050; Linked HERO ID(s): 3345050, 5253108

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome adequately.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	The test organisms were reported to be acclimated, but the test conditions were not reported in great detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	It was reported that there were no mortalities, so statistical analysis of the mortality was not necessary.
	Metric 22: Reporting of Data	Low	Mortality results were reported for the exposure levels and the controls in the text only.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation is for the mortality of *Catla catla* after exposure to 1,2-DCB after a 28d exposure. Fish were sampled at 24h and at the termination of the test.

Overall Quality Determination

Medium

Study Citation:	Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, <i>Catla catla</i> L. using alkaline comet assay. <i>Bulletin of Environmental Contamination and Toxicology</i> 91(6):616-622.
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; <i>Catla catla</i> L.; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	o-Dichlorobenzene
HERO ID:	3345050; Linked HERO ID(s): 3345050, 5253108

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test 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 percent purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	An appropriate solvent control was reported.
	Metric 5: Negative Control Response	High	No mortality was reported, and the control response to DNA damage was reported and appropriate.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations and/or minimize loss of the test substance before and during the exposure.
	Metric 8: Consistency of Exposure Administration	Low	Details of exposure administration were not reported, making it difficult to assess consistency.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were only measured in the highest concentration/stock solution.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified for a dose response by study authors. They were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	Medium	The solvent concentration was not reported, but the exposure levels were well below the solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from a local farm, but an actual name was not reported. The age of the fish was also not reported, but the lengths and the weights were reported.
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for 10 days prior to the start of the study.
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test replicates was not reported.
Domain 5: Outcome Assessment			

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Study Citation: Ganesan, N., Sathya, T. N., Arunachalam, K. D. (2013). Genotoxicity evaluation of 1,2 dichlorobenzene in the Indian major carp, *Catla catla* L. using alkaline comet assay. *Bulletin of Environmental Contamination and Toxicology* 91(6):616-622.

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; *Catla catla* L.; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mechanistic-Genotox (including DNA repair)

Chemical: o-Dichlorobenzene

HERO ID: 3345050; Linked HERO ID(s): 3345050, 5253108

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not sufficiently reported to evaluate if they were adequate.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome adequately.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	The test organisms were reported to be acclimated, but the test conditions were not reported in great detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described and were appropriate for the dataset.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation is for the genotoxic effects found in the gills and blood of *Catla catla* after exposure to 1,2-DCB after a 28d exposure. Fish were sampled at 24h and at the termination of the test.

Overall Quality Determination

Medium

Study Citation:	Zhao, Y. H., Wang, L. S., Gao, H., Zhang, Z. (1993). Quantitative structure–activity relationships: Relationship between toxicity of organic chemicals to fish and to photobacterium phosphoreum. <i>Chemosphere</i> 26(11):1971-1979.			
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; <i>Cyprinus carpio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	647011			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	No source or manufacturer was provided.	
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	No data was provided on the use of a negative control.	
	Metric 5: Negative Control Response	Uninformative	Survival within any possible control group(s) was not reported.	
	Metric 6: Randomized Allocation	Low	The study did not report any randomized allocation.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study did not adequately report the solution preparation. It also did not detail the volume of acetone used as a carrier for the test substance.	
	Metric 8: Consistency of Exposure Administration	Uninformative	The gradient of exposure concentrations (nominal or actual) were not listed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	This study used a 48h duration for acute toxicity.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was reported for exposure concentrations.	
	Metric 12: Testing at or Below Solubility Limit	Uninformative	Compound concentrations were not provided to determine if the solubility limit was reached.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The size of the animal was approximated at "5g and length 5cm," and the source of the fish was a commercial supplier.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	A two week acclimation was reported, however, it was only described as "laboratory conditions" with no details on what those settings were.	
	Metric 15: Number of Organisms and Replicates per Group	Low	10 fish were used per treatment concentration, but no replicates were reported. 50 individuals per test were reported, but there were no details on treatment groups.	
Domain 5: Outcome Assessment				
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Study Citation:	Zhao, Y. H., Wang, L. S., Gao, H., Zhang, Z. (1993). Quantitative structure–activity relationships: Relationship between toxicity of organic chemicals to fish and to photobacterium phosphoreum. <i>Chemosphere</i> 26(11):1971-1979.
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; <i>Cyprinus carpio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	647011

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	This study has a loading biomass of 3.1g/L compared to EPA 850 guidelines for 0.8 g/L. No water quality or critical animal care conditions were reported before or during the bioassay.
	Metric 17: Outcome Assessment Methodology	Low	The methods section did not provide indication that replication was used in this bioassay or if and how often dead animals were removed from test chambers.
	Metric 18: Consistency of Outcome Assessment	Low	The methodology was not sufficient to report when live/dead observations were made throughout the bioassay, stating "numbers of live and dead were determined on a regular schedule."
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	No environmental conditions were reported to ensure animal care was reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information on outcomes was reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was not described for the formulation of LC50s.
	Metric 22: Reporting of Data	Low	LC50 data for o-Dichlorobenzene are as log1/LC50. No other information on the dose-response from this bioassay was provided.
	Metric 23: Explanation of Unexpected Outcomes	Low	Data was provided without measures of variability.
Additional Comments:	This study does not provide adequate information of the test substance concentrations (solubility limit concerns), has limited statistical power with no replication, and does not provide dose-response data other than report a log1/LC50 for o-Dichlorobenzene.		

Overall Quality Determination

Uninformative

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. <i>Environmental Toxicology and Chemistry</i> 22(2):329-335.		
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; <i>Danio rerio</i> ; Adult		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	1417745		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was identified as Sigma Aldrich, but it was not reported to be analytically verified.
	Metric 3: Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls were reported for this study.
	Metric 5: Negative Control Response	Low	Control mortality for the 96h acute test was not reported. Only LC50 values were reported.
	Metric 6: Randomized Allocation	Low	Organism allocation was not detailed.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was well detailed, however, the use of a static assay without renewal complicates the consistent exposure of the test compound to the animals in this 96h bioassay.
	Metric 8: Consistency of Exposure Administration	Medium	The use of solvent controls and exposure conditions appeared consistent across treatment groups. The static renewal nature of this bioassay may have created issues with exposure consistency.
	Metric 9: Measurement of Test Substance Concentration	Low	Water was not tested throughout the 96-hr bioassay, however, a daily test of water from 0, 48, and 72h after mixing was completed for a different (chronic) bioassay in this paper. This work (Table 1) indicated significant declines in compound concentration throughout time. The results in the current acute study were reported as nominal.
	Metric 10: Exposure Duration and Frequency	High	The 96-hr acute duration for this acute bioassay is appropriate.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	This study used a solvent control and three concentrations (56, 100, and 180 mg/L) which has unequal spacing and not enough treatment concentrations for good resolution of an LC50 value.
	Metric 12: Testing at or Below Solubility Limit	Medium	The highest nominal concentration reported (180 mg/l) was slightly above the solubility reported in the Final Scope for o-DCB (156 mg/l). Ethanol was reported to be used to aid in dissolution.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.
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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.		
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; <i>Danio rerio</i> ; Adult		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	1417745		
Domain	Metric	Rating	Comments
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 5 males and 5 females per tank. This is a relatively low number of organisms per treatment.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Biomass loading was unacceptable for this bioassay. With a 2.5 liter tank and 10 fish with an average mass of 0.8g each, the biomass would be 3.2g/l which exceeds the EPA 850 guidelines for 0.8 g/l. Ammonia production throughout this static assay period could significantly influence the estimation of an LC50 for this compound.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Water quality throughout the bioassay was not reported, and the biomass loading at 3.2 g/l raises serious concerns with water conditions throughout a 96-hr static test.
	Metric 20: Outcomes Unrelated to Exposure	Medium	Nothing in the study indicated that animal attrition of health outcomes were present among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this acute bioassay.
	Metric 22: Reporting of Data	Low	Authors only provided the estimated LC50s for this acute assay. Individual concentration results and control results were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Medium	No variance was reported because the outcomes for acute bioassay indicated that the LC50 was above the highest treatment concentration tested (180 mg/L).
Additional Comments:	The use of 2.5 l tanks for a 96-hr bioassay with 10 adult zebrafish is way above guidance for biomass loading. In addition, this study provides nominal concentrations and showed significant loss of treatment concentrations overtime. No renewal of treatment concentrations is a concern across the 96-hr study duration. The bioassay only had three treatment groups with unequal spacing.		

Overall Quality Determination**Low**

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. <i>Environmental Toxicology and Chemistry</i> 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
Metric 2:	Test Substance Source	Low	The source/manufacturer was identified (Sigma-Aldrich), but it was not reported to be analytically verified.
Metric 3:	Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Negative controls (with the ethanol solvent) were reported for this study. Positive controls (using 5, 50 ng/L of estradiol) were also included.
Metric 5:	Negative Control Response	High	The negative control response was reported in Table 3.
Metric 6:	Randomized Allocation	Low	Organism allocation was not detailed.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Renewal was performed every 72 hours which is a concern due to the rapid loss of the test compound from 0 to 72 hours based on the study's reporting of 0, 48, and 72 hour change in the compound.
Metric 8:	Consistency of Exposure Administration	Low	The use of solvent controls and exposure conditions appeared consistent across treatment groups. However, the loss of test substance over time creates concern regarding consistency.
Metric 9:	Measurement of Test Substance Concentration	Low	Water was not tested throughout this subchronic 21 day bioassay; however, a daily test of water from 0, 48, and 72 hr after mixing indicated significant declines in compound concentration throughout time. The results in the current subchronic study were reported as nominal.
Metric 10:	Exposure Duration and Frequency	High	The 14-day exposure period for this subchronic assay on vitellogenin response is appropriate.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two concentrations of o-DCB were examined in this subchronic bioassay (0.1, 1 mg/L), which is a narrow range for an examination of a potential vitellogenin response.
Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations are within the solubility limits for this compound.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.

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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 10 males and 10 females per tank. This is lower than is typical.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	The test tank volume for this subchronic bioassay was not described, so biomass loading calculations were not possible. Dissolved oxygen was monitored, and water was renewed every 3 days.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Water quality throughout the 14 day study was not reported other than dissolved oxygen. Tank volume was not reported.
	Metric 20: Outcomes Unrelated to Exposure	High	Nothing in the study indicated that animal attrition of health outcomes were present among groups. Negative and positive control groups for both males and females were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this subchronic bioassay.
	Metric 22: Reporting of Data	High	Calculations of condition factor, hepatosomatic index, gonadosomatic index, and % VTG in blood protein was reported for each treatment group and between male and female groups in Table 3.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The sample numbers within each treatment by fish sex are such that the statistical analysis for these data have confidence in the results.
Additional Comments:	Replication in this study and the use of both positive and negative controls builds confidence in the results. Tank volume was not reported, and a 72-hr renewal for this 14 day study could lower the confidence. Nominal concentrations were used in reporting even though there was substantial loss of test substance.		

Overall Quality Determination

Medium

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.			
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult			
Health Outcome:	Hepatic/Liver			
Chemical:	o-Dichlorobenzene			
HERO ID:	1417745			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source/manufacturer was identified (Sigma-Aldrich), but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (with the ethanol solvent) were reported for this study. Positive controls (using 5, 50 ng/L of estradiol) were also included.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Table 3.	
	Metric 6: Randomized Allocation	Low	Organism allocation was not detailed.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Renewal was performed every 72 hours which is a concern due to the rapid loss of the test compound from 0 to 72 hours based on the study's reporting of 0, 48, and 72 hour change in the compound.	
	Metric 8: Consistency of Exposure Administration	Low	The use of solvent controls and exposure conditions appeared consistent across treatment groups. However, the loss of test substance over time creates concern regarding consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	Water was not tested throughout this subchronic 21 day bioassay; however, a daily test of water from 0, 48, and 72 hr after mixing indicated significant declines in compound concentration throughout time. The results in the current subchronic study were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The 14-day exposure period was appropriate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two concentrations of o-DCB were examined in this subchronic bioassay (0.1, 1 mg/L), which is a narrow range.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentrations are within the solubility limits for this compound.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 10 males and 10 females per tank. This is lower than is typical.	
Domain 5: Outcome Assessment				

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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Hepatic/Liver
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	The test tank volume for this subchronic bioassay was not described, so biomass loading calculations were not possible. Dissolved oxygen was monitored, and water was renewed every 3 days.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Water quality throughout the 14 day study was not reported other than dissolved oxygen. Tank volume was not reported.
	Metric 20: Outcomes Unrelated to Exposure	High	Nothing in the study indicated that animal attrition of health outcomes were present among groups. Negative and positive control groups for both males and females were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this subchronic bioassay.
	Metric 22: Reporting of Data	High	Calculations of condition factor, hepatosomatic index, gonadosomatic index, and % VTG in blood protein was reported for each treatment group and between male and female groups in Table 3.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The sample numbers within each treatment by fish sex are such that the statistical analysis for these data have confidence in the results.
Additional Comments:	Replication in this study and the use of both positive and negative controls builds confidence in the results. Tank volume was not reported, and a 72-hr renewal for this 14 day study could lower the confidence. Nominal concentrations were used in reporting even though there was substantial loss of test substance. This form is for the hepatosomatic index, which is a hepatic/liver outcome.		

Overall Quality Determination

Medium

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.			
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult			
Health Outcome:	Reproductive/Teratogenic			
Chemical:	o-Dichlorobenzene			
HERO ID:	1417745			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source/manufacturer was identified (Sigma-Aldrich), but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (with the ethanol solvent) were reported for this study. Positive controls (using 5, 50 ng/L of estradiol) were also included.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Table 3.	
	Metric 6: Randomized Allocation	Low	Organism allocation was not detailed.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Renewal was performed every 72 hours which is a concern due to the rapid loss of the test compound from 0 to 72 hours based on the study's reporting of 0, 48, and 72 hour change in the compound.	
	Metric 8: Consistency of Exposure Administration	Low	The use of solvent controls and exposure conditions appeared consistent across treatment groups. However, the loss of test substance over time creates concern regarding consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	Water was not tested throughout this subchronic 21 day bioassay; however, a daily test of water from 0, 48, and 72 hr after mixing indicated significant declines in compound concentration throughout time. The results in the current subchronic study were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The 14-day exposure period was appropriate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two concentrations of o-DCB were examined in this subchronic bioassay (0.1, 1 mg/L), which is a narrow range.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentrations are within the solubility limits for this compound.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 10 males and 10 females per tank. This is lower than is typical.	
Domain 5: Outcome Assessment				

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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	The test tank volume for this subchronic bioassay was not described, so biomass loading calculations were not possible. Dissolved oxygen was monitored, and water was renewed every 3 days.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Water quality throughout the 14 day study was not reported other than dissolved oxygen. Tank volume was not reported.
	Metric 20: Outcomes Unrelated to Exposure	High	Nothing in the study indicated that animal attrition of health outcomes were present among groups. Negative and positive control groups for both males and females were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this subchronic bioassay.
	Metric 22: Reporting of Data	High	Calculations of condition factor, hepatosomatic index, gonadosomatic index, and % VTG in blood protein was reported for each treatment group and between male and female groups in Table 3.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The sample numbers within each treatment by fish sex are such that the statistical analysis for these data have confidence in the results.
Additional Comments:	Replication in this study and the use of both positive and negative controls builds confidence in the results. Tank volume was not reported, and a 72-hr renewal for this 14 day study could lower the confidence. Nominal concentrations were used in reporting even though there was substantial loss of test substance. This form is for the gonadosomatic index, which is a reproductive/teratogenic outcome.		

Overall Quality Determination

Medium

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.			
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	1417745			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source/manufacturer was identified (Sigma-Aldrich), but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (with the ethanol solvent) were reported for this study. Positive controls (using 5, 50 ng/L of estradiol) were also included.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Table 3.	
	Metric 6: Randomized Allocation	Low	Organism allocation was not detailed.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Renewal was performed every 72 hours which is a concern due to the rapid loss of the test compound from 0 to 72 hours based on the study's reporting of 0, 48, and 72 hour change in the compound.	
	Metric 8: Consistency of Exposure Administration	Low	The use of solvent controls and exposure conditions appeared consistent across treatment groups. However, the loss of test substance over time creates concern regarding consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	Water was not tested throughout this subchronic 21 day bioassay; however, a daily test of water from 0, 48, and 72 hr after mixing indicated significant declines in compound concentration throughout time. The results in the current subchronic study were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The 14-day exposure period was appropriate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two concentrations of o-DCB were examined in this subchronic bioassay (0.1, 1 mg/L), which is a narrow range.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentrations are within the solubility limits for this compound.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 10 males and 10 females per tank. This is lower than is typical.	
Domain 5: Outcome Assessment				

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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	The test tank volume for this subchronic bioassay was not described, so biomass loading calculations were not possible. Dissolved oxygen was monitored, and water was renewed every 3 days.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Water quality throughout the 14 day study was not reported other than dissolved oxygen. Tank volume was not reported.
	Metric 20: Outcomes Unrelated to Exposure	High	Nothing in the study indicated that animal attrition of health outcomes were present among groups. Negative and positive control groups for both males and females were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this subchronic bioassay.
	Metric 22: Reporting of Data	High	Calculations of condition factor, hepatosomatic index, gonadosomatic index, and % VTG in blood protein was reported for each treatment group and between male and female groups in Table 3.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The sample numbers within each treatment by fish sex are such that the statistical analysis for these data have confidence in the results.

Additional Comments: Replication in this study and the use of both positive and negative controls builds confidence in the results. Tank volume was not reported, and a 72-hr renewal for this 14 day study could lower the confidence. Nominal concentrations were used in reporting even though there was substantial loss of test substance. This form is for the condition factor, which is a development/growth outcome.

Overall Quality Determination

Medium

Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. <i>Environmental Toxicology and Chemistry</i> 22(2):329-335.			
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	1417745			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source/manufacturer was identified (Sigma-Aldrich), but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported as it comes from Sigma-Aldrich (neat standard 99%).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (with the ethanol solvent) were reported for this study. Positive controls (using 5, 50 ng/L of estradiol) were also included.	
	Metric 5: Negative Control Response	High	The negative control response was reported in Table 3.	
	Metric 6: Randomized Allocation	Low	Organism allocation was not detailed.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Renewal was performed every 72 hours which is a concern due to the rapid loss of the test compound from 0 to 72 hours based on the study's reporting of 0, 48, and 72 hour change in the compound.	
	Metric 8: Consistency of Exposure Administration	Low	The use of solvent controls and exposure conditions appeared consistent across treatment groups. However, the loss of test substance over time creates concern regarding consistency.	
	Metric 9: Measurement of Test Substance Concentration	Low	Water was not tested throughout this subchronic 21 day bioassay; however, a daily test of water from 0, 48, and 72 hr after mixing indicated significant declines in compound concentration throughout time. The results in the current subchronic study were reported as nominal.	
	Metric 10: Exposure Duration and Frequency	High	The 14-day exposure period was appropriate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Two concentrations of o-DCB were examined in this subchronic bioassay (0.1, 1 mg/L), which is a narrow range.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentrations are within the solubility limits for this compound.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source, sex, length, and weight of the test animals were adequately reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Water quality and tank volumes were reported for these fish before the bioassay, however, the duration of this period before testing was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Each control and treatment concentration consisted of 2 replicates with 10 males and 10 females per tank. This is lower than is typical.	
Domain 5: Outcome Assessment				

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Study Citation:	Versonnen, B. J., Arijs, K., Verslycke, T., Lema, W., Janssen, C. R. (2003). In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. Environmental Toxicology and Chemistry 22(2):329-335.
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:	Vertebrate; Fish; <i>Danio rerio</i> ; Adult
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	1417745

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	The test tank volume for this subchronic bioassay was not described, so biomass loading calculations were not possible. Dissolved oxygen was monitored, and water was renewed every 3 days.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methods are described and appropriate.
	Metric 18: Consistency of Outcome Assessment	High	There is consistency in outcome assessment among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Water quality throughout the 14 day study was not reported other than dissolved oxygen. Tank volume was not reported.
	Metric 20: Outcomes Unrelated to Exposure	High	Nothing in the study indicated that animal attrition of health outcomes were present among groups. Negative and positive control groups for both males and females were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was reported for this subchronic bioassay.
	Metric 22: Reporting of Data	High	Calculations of condition factor, hepatosomatic index, gonadosomatic index, and % VTG in blood protein was reported for each treatment group and between male and female groups in Table 3.
	Metric 23: Explanation of Unexpected Outcomes	Medium	The sample numbers within each treatment by fish sex are such that the statistical analysis for these data have confidence in the results.
Additional Comments:	Replication in this study and the use of both positive and negative controls builds confidence in the results. Tank volume was not reported, and a 72-hr renewal for this 14 day study could lower the confidence. Nominal concentrations were used in reporting even though there was substantial loss of test substance. This form is for the mortality outcome.		

Overall Quality Determination

Medium

Study Citation:	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (<i>Lepomis macrochirus</i>). Bulletin of Environmental Contamination and Toxicology 26(4):446-452.
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; <i>Lepomis macrochirus</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	18064; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Only the chemical name was given.
	Metric 2: Test Substance Source	Low	The exact source was not indicated. Authors mentioned that the test chemicals were obtained from commercial sources that were able to provide the purest grade available. It was not reported whether the test chemical was analytically verified.
	Metric 3: Test Substance Purity	Medium	Authors reported the test chemical purity was equal to or greater than 80%, but an exact value was not given.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A negative control was used. Since Table 1 shows that o-DCB was soluble it is assumed no solvent carrier was used. This was also explained in the text under 'Materials and Methods'.
	Metric 5: Negative Control Response	Low	Mortality of the control group was not reported.
	Metric 6: Randomized Allocation	Medium	Fish were reported to be randomly selected from the test population and placed in each test jar.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system was adequately described. Test media preparation was vague. Test concentrations were not measured, and volatilization could have occurred.
	Metric 8: Consistency of Exposure Administration	Low	Since details were lacking on test solution preparation and how many test concentrations were even used it is unclear if exposure administration was consistent. Test concentrations were not measured, and volatilization could have occurred.
	Metric 9: Measurement of Test Substance Concentration	Low	Test concentrations were not reported. The data reported was based on nominal test concentrations.
	Metric 10: Exposure Duration and Frequency	High	The 96-hour duration was appropriate for an acute static fish toxicity test.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Uninformative	The test concentrations used were not reported by study authors. Authors reported using low, medium, and high test concentrations but no values were given.
	Metric 12: Testing at or Below Solubility Limit	Medium	Since Table 1 shows that o-DCB was soluble it is assumed no solvent carrier was used. This means that the test concentrations used were probably below the water solubility limit. However, since the actual test concentration values were not reported, one can't say for certain.
Domain 4: Test Organism			

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Study Citation:	Buccafusco, R. J., Ells, S. J., Leblanc, G. A. (1981). Acute toxicity of priority pollutants to bluegill (<i>Lepomis macrochirus</i>). Bulletin of Environmental Contamination and Toxicology 26(4):446-452.			
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; <i>Lepomis macrochirus</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	18064; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	The source was vaguely described. Authors just mentioned the fish were obtained from commercial fish suppliers within the US. Age and size of the fish were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Fish were acclimated to test conditions for 48 hours prior to test start.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Ten fish were placed in each test jar. No replicates were reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Acclimation and test conditions were reported and adequate, except for dissolved oxygen. Authors did mention that dissolved oxygen concentrations dropped significantly by the end of the exposure.	
	Metric 17: Outcome Assessment Methodology	High	Authors mentioned assessing mortality throughout the test.	
	Metric 18: Consistency of Outcome Assessment	High	Mortality was assessed at the start of the test and every 24 hours during the exposure (24, 48, and 96 hours).	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Fish were acclimated to test conditions prior to start. Test conditions were measured consistently throughout the exposure. Authors did mention that dissolved oxygen concentrations dropped significantly by the end of the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences in fish between groups that could impact the outcome assessment.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical analyses used were adequately described.	
	Metric 22: Reporting of Data	Low	Only 24 and 96-hour LC50s were reported. No raw data was shown. Also, authors mentioned that the LC50 values were calculated based off nominal concentrations and not actual measured test concentrations.	
	Metric 23: Explanation of Unexpected Outcomes	Medium	There were no measures of variability shown with the 24-hour LC50 value. The numbers in parentheses for the 96-hour LC50 value may represent measures of variability but it is not certain.	

Additional Comments: This evaluation is for the assessment of mortality. This evaluation received an unacceptable rating because the test concentrations used were not reported.

Overall Quality Determination

Uninformative

Study Citation:	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (<i>Lepomis macrochirus</i>). :379-392.
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:	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	18050; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test chemical was identified by name only. The position of the 14C label was reported, but no other identifying information was reported.
Metric 2:	Test Substance Source	Low	It was reported that the test chemicals were from New England Nuclear in Boston, MA, but it was not reported to be analytically verified.
Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate negative control in which the test systems only received well water.
Metric 5:	Negative Control Response	Low	The negative control response was not reported. Only BCF values were reported.
Metric 6:	Randomized Allocation	Low	It was not reported how the bluegill were allocated to study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The test system was a flow-through system that used a continual-flow proportional diluter. A closed system was used for volatile compounds. The stock solution preparation was described.
Metric 8:	Consistency of Exposure Administration	Medium	The exposure administration appears to be consistent across all study groups. Test chambers and conditions appeared to be similar across study groups. However, four populations of fish were used for this study. Three of these were from the same source, while the other was from a different source. Sizes appeared to be similar, but it was not reported if the the different populations were distributed evenly through the test. This could affect the results.
Metric 9:	Measurement of Test Substance Concentration	High	Water was sampled on a regular basis during the test period. Mean water concentrations are reported in Table III.
Metric 10:	Exposure Duration and Frequency	High	The exposure period for the DCBs was reported to be 14 days with a 7-day depuration period. This appeared adequate for the outcome of interest.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There appears to only be one exposure group to determine the BCF. The mean water concentration is reported in Table III.
Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was below the water solubility limit.
Domain 4: Test Organism			

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Study Citation:	Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (<i>Lepomis macrochirus</i>). :379-392.			
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:	Vertebrate; Fish; <i>Lepomis macrochirus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	18050; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	Four populations of bluegills were used in this test. Three of those were from the same commercial fish farmer in Connecticut. One population was from a commercial fish farmer in Nebraska. Fish appeared to be similar in length and weight, the different sources creates minor reservations. It is unclear how the different fish were distributed to test groups.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The fish were acclimated for 30 days prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that 100 bluegill were placed into an aquarium of the appropriate test system. It is unclear if all 100 fish were placed in one aquarium or if the fish were distributed to appropriate test/control aquariums. The number of replicates was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	The pH, DO, and temperature were reported and appeared appropriate for the test organisms. The fish were reported to be fed ad libitum three times a week for the duration of the study. Little information was provided on the dilution water characteristics.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—BCF of the test compound in the fish after 14 days of exposure.	
	Metric 18: Consistency of Outcome Assessment	High	Methods for outcome assessment were reported and appeared to be consistent.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	The fish were acclimated to test conditions prior to the start of the test, and test conditions were described adequately. There did not appear to be differences among study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Little information was provided on the determination of the BCF values.	
	Metric 22: Reporting of Data	Low	BCF values were reported for each of the chemicals tested. There did not appear to be any report on control results.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Measures of variability were provided in Table III.	
Additional Comments:	This evaluation is for the BCF value for o-DCB in <i>L. macrochirus</i> .			

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Study Citation: Barrows, M. E., Petrocelli, S. R., Macek, K. J., Carroll, J. J. (1980). Bioconcentration and elimination of selected water pollutants by bluegill sunfish (*Lepomis macrochirus*). :379-392.

Duration: Overall 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; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: ADME (biotransformation)

Chemical: o-Dichlorobenzene

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

Domain	Metric	Rating	Comments
Overall Quality Determination		Medium	

Study Citation:	Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Morairity, A., Richter, J. (1984). Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – parts I and II.			
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; <i>Oncorhynchus mykiss</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	7494661; Linked HERO ID(s): 3634370, 7494661			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.	
Metric 2:	Test Substance Source	Low	The source of chemicals was not stated for the rainbow trout acute toxicity test, and analytical confirmation of the test substance was not reported.	
Metric 3:	Test Substance Purity	Low	Purity of the test substance was not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported that all tests had a concurrent negative control.	
Metric 5:	Negative Control Response	Low	The negative control response was not reported.	
Metric 6:	Randomized Allocation	Low	It was not reported how the rainbow trout were allocated into study groups.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	The exposure system was reported to be a flow-through diluter system. The test media preparation was not reported, but concentrations were measured during the study. Recovery for the concentration range was reported to be 103.7 +/- 2.6%.	
Metric 8:	Consistency of Exposure Administration	High	Test chamber dimensions were the same for all tanks in the study with dimensions of 21.0 x 35.0 x 24.5 cm. The chambers were filled with 9.0 cm of test solution. Loading ranged from 1.3 to 4.1 g/L.	
Metric 9:	Measurement of Test Substance Concentration	Medium	Test concentrations were measured in all chambers at the start of the test and at 96h. during the test, one chamber of each replicate was measured for toxicant concentration. o-Dichlorobenzene was measured using GLC. Recovery for the range of concentrations was reported to be 103.7 +/- 2.6%, indicating that measured concentrations were similar to nominal concentrations.	
Metric 10:	Exposure Duration and Frequency	High	The study duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups, and spacing appeared adequate to obtain a 96h LC50 value.	
Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Medium	The test organisms were reported to be from Lake Mills, Wisconsin, National Fish Hatchery. The age was not reported for this test chemical, but the mean length and weight were reported.	

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Study Citation:	Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Morairity, A., Richter, J. (1984). Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – parts I and II.
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; <i>Oncorhynchus mykiss</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	7494661; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions.
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 fish per chamber and two replicates per concentration, which is the preferred replication in the OCSPP 850.1075 guideline.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The room temperature was held at 12C, and the photoperiod was 16h light. It was reported that temperature, pH, and DO were monitored during the test, but only ranges across all chemicals tested were reported. The ranges across all chemicals appeared adequate. It was not reported if the fish were fed at any point prior to the study or during the study.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality in the form of a 96h LC50 value.
	Metric 18: Consistency of Outcome Assessment	High	Mortalities were monitored at 1, 2, 4, 8, 12, and 24h and then daily after that point.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	LC50 values were determined using the trimmed Spearman-Kärber method.
	Metric 22: Reporting of Data	Low	Only LC50 and EC50 values were reported. Data were not reported for each test concentration and control.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.
Additional Comments:	This evaluation is for the acute toxicity of o-dichlorobenzene on rainbow trout. The age of the trout was not reported for this test, but weight and length were reported. 96h LC50 values were reported, and mortality was the outcome of interest.		

Overall Quality Determination**Medium**

Study Citation:	Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Morairity, A., Richter, J. (1984). Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – parts I and II.		
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; <i>Oncorhynchus mykiss</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Behavioral		
Chemical:	o-Dichlorobenzene		
HERO ID:	7494661; Linked HERO ID(s): 3634370, 7494661		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of chemicals was not stated for the rainbow trout acute toxicity test, and analytical confirmation of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	Purity of the test substance was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported that all tests had a concurrent negative control.
	Metric 5: Negative Control Response	Low	The negative control response was not reported.
	Metric 6: Randomized Allocation	Low	It was not reported how the rainbow trout were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The exposure system was reported to be a flow-through diluter system. The test media preparation was not reported, but concentrations were measured during the study. Recovery for the concentration range was reported to be 103.7 +/- 2.6%.
	Metric 8: Consistency of Exposure Administration	High	Test chamber dimensions were the same for all tanks in the study with dimensions of 21.0 x 35.0 x 24.5 cm. The chambers were filled with 9.0 cm of test solution. Loading ranged from 1.3 to 4.1 g/L.
	Metric 9: Measurement of Test Substance Concentration	Medium	Test concentrations were measured in all chambers at the start of the test and at 96h. during the test, one chamber of each replicate was measured for toxicant concentration. o-Dichlorobenzene was measured using GLC. Recovery for the range of concentrations was reported to be 103.7 +/- 2.6%, indicating that measured concentrations were similar to nominal concentrations.
	Metric 10: Exposure Duration and Frequency	High	The study duration as reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups, and spacing appeared adequate to obtain a 96h EC50 value.
	Metric 12: Testing at or Below Solubility Limit	High	All test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from Lake Mills, Wisconsin, National Fish Hatchery. The age was not reported for this test chemical, but the mean length and weight were reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions.
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Study Citation:	Ahmad, N., Benoit, D., Brooke, L., Call, D., Carlson, A., DeFoe, D., Huot, J., Morairity, A., Richter, J. (1984). Aquatic toxicity tests to characterise the hazard of volatile organic chemicals in water: A toxicity data summary – parts I and II.
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; <i>Oncorhynchus mykiss</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Behavioral
Chemical:	o-Dichlorobenzene
HERO ID:	7494661; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 fish per chamber and two replicates per concentration, which is the preferred replication in the OCSPP 850.1075 guideline.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The room temperature was held at 12C, and the photoperiod was 16h light. It was reported that temperature, pH, and DO were monitored during the test, but only ranges across all chemicals tested were reported. The ranges across all chemicals appeared adequate. It was not reported if the fish were fed at any point prior to the study or during the study.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—swimming behavior. An EC50 value was determined based on behavioral observations.
	Metric 18: Consistency of Outcome Assessment	Low	It was unclear when behavioral assessments were made during the study, whether those occurred at the same time as mortality assessments or at a different time interval.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	The method for determining EC50 values for behavioral responses was not reported.
	Metric 22: Reporting of Data	Low	Only LC50 and EC50 values were reported. Data were not reported for each test concentration and control.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

Additional Comments: This evaluation is for the acute toxicity of o-dichlorobenzene on rainbow trout. The age of the trout was not reported for this test, but weight and length were reported. 96h EC50 values were reported for changes in behavior due to chemical exposure.

Overall Quality Determination

Medium

Study Citation:	Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. <i>Water Research</i> 13(2):137-141.			
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; <i>Pimephales promelas</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	1392282; Linked HERO ID(s): 1392282, 5352394			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	Source was not reported, and the chemical identity not analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were used.	
	Metric 5: Negative Control Response	High	Mortality in the controls was less than 10%, as reported in the text.	
	Metric 6: Randomized Allocation	Low	The study does not report random allocation of test organisms.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study does not provide sufficient details of test media preparation for testing the compound of interest.	
	Metric 8: Consistency of Exposure Administration	Uninformative	In the results section, it is stated that o-dichlorobenzene was administered in crystal form and remained undissolved.	
	Metric 9: Measurement of Test Substance Concentration	Low	Nominal concentrations and exposure concentrations (though measured) were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The 96 hr acute toxicity test was appropriate for the outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups.	
	Metric 12: Testing at or Below Solubility Limit	Low	Nominal and exposure concentrations were not reported and cannot determine whether exposure concentration exceeded the solubility limit. It was reported that the test chemical was not fully dissolved and it was "visibly insoluble at the ranges tested."	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the test organisms and the size were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The test organisms were acclimatized but it cannot be determined if the pretreatment conditions were the same for the control and the exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 5 animals per replicate and 2 replicates per concentration.	
Domain 5: Outcome Assessment				

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Study Citation: Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. *Water Research* 13(2):137-141.
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*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 1392282; Linked HERO ID(s): 1392282, 5352394

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions were recorded during the test but results were not reported. Temperature was reported to be 22C in both test waters. The reconstituted dilution water characteristics were reported as well. Organisms were fed during holding and not fed for the test, which is typical. DO and temperature were monitored daily for the test.
	Metric 17: Outcome Assessment Methodology	High	The mortality outcome assessment was appropriate for the test.
	Metric 18: Consistency of Outcome Assessment	Medium	Mortality was checked at 24, 48, and 96 hours, but does not provide details to assess whether this protocol was consistent for control and treatment groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study does not report the data on environmental conditions recorded during the test.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences in the outcome unrelated to the exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	LC 50 values and the confidence intervals were determined using probit analysis.
	Metric 22: Reporting of Data	Low	No data was reported on nominal or exposure concentrations, mortality outcomes for each concentration, or environmental conditions. Only LC 50 values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.
Additional Comments:	This evaluation is for o-DCB in freshwater. The study did not report the exposure or nominal concentrations, the number of exposure groups, environmental conditions the of controls and the treatment groups during the test, or the dose-response data. Only LC 50 values were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Skin & Connective Tissue		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN.
	Metric 2: Test Substance Source	Low	The o-DCB was reported to be from Aldrich Chemical Co. It was not reported if the test substance was analytically verified though it was noted that one of three techniques was used to analyze toxicants in water samples from fish exposure tanks.
	Metric 3: Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	The response of the negative control was reported and was adequate for the outcome of interest. This was reported as the "effect" outcome.
	Metric 6: Randomized Allocation	Medium	It was reported that fish were removed from a common pool and distributed at random among exposure chambers.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test chemicals were prepared using a variety of methods: diluted stock, emulsified stock, liquid-liquid equilibrator, and glass wool equilibrator depending on the test substance characteristic. An 84mg/L stock was prepared by diluting stock where blending the test substance vigorously was the mixing method. A flow-through system was reported using one of three systems: a continuous-flow modified min-diluter, an ABC solenoid operated electronic diluter, or a single-cell glass column system.
	Metric 8: Consistency of Exposure Administration	Medium	Several test chemicals were assessed for this reference. There were slight minor variations in how the tests were conducted based on the year the tests were conducted and the location testing occurred (not all tests were conducted in the same laboratory). Fish were either cultured at U.S. EPA or at the University of Wisconsin-Superior depending on the chemical tested. These were minor conditions that likely would not greatly affect the outcome of the studies. Testing for o-DCB itself and its administration appeared consistent across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Gas-liquid chromatography was used to measure test concentrations. Tests were sampled at 0, 24, 48, 72, and 96h, and all samples were analyzed immediately or preserved for later analysis.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five test concentration, and spacing was adequate to see a dose response.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.			
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; <i>Pimephales promelas</i> ; Juvenile			
Health Outcome:	Skin & Connective Tissue			
Chemical:	o-Dichlorobenzene			
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	High	All test concentrations were well below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The fathead minnows were reported to be 32d of age. All test fish were cultured at the U.S. EPA Environmental Research Laboratory in Duluth, MN and at the University of Wisconsin-Superior campus.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	It was reported that embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Culture temperature and photoperiod were also the same as test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 20 fish per test treatment, but the number of replicates was unclear.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Temperature, D.O., hardness, alkalinity, and pH were all reported and monitored. Culture conditions were similar to test conditions, and fish were fed brine shrimp during culturing. A 16L:8D photoperiod was maintained during culturing and for the duration of the test period.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—signs of toxicity including pigmentation changes and deformations.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The number of dead fish was noted every 24h after the beginning of the test. Changes in behavior and signs of toxicity (changes in pigmentation, deformations, etc.) were noted at 2-8, 24, 48, 72, and 96h.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Culture conditions and test conditions were described in detail.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate for the outcomes of interest.

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Study Citation: Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): Volume III. :328.
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*; Juvenile
Health Outcome: Skin & Connective Tissue
Chemical: o-Dichlorobenzene
HERO ID: 32170; Linked HERO ID(s): 32170, 3660853

Domain	Metric	Rating	Comments
	Metric 22: Reporting of Data	Medium	Data for o-DCB outcomes was reported in the document 111/112 (p130/347 in the PDF). Data for the control response and the exposure response were reported here along with the estimated 96h EC50 value. Response curves were also reported here. Note that the changes in skin pigmentation are reported as the "effect" outcome. It is not specified how many fish displayed changes in pigmentation, but signs of toxicity were noted in the "Remarks" section.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report any unexpected outcomes. Confidence limits for the EC50 value were not reported.

Additional Comments: This evaluation is for the acute toxicity of o-DCB on juvenile fathead minnows. An EC50 value was reported for changes in behavior (evaluated separately) due to toxicity. Other contributing factors in determining the EC50 include skin pigmentation and reported deformation (evaluated separately). This evaluation is for the changes in skin pigmentation reported in the remarks.

Overall Quality Determination

High

Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN.
	Metric 2: Test Substance Source	Low	The o-DCB was reported to be from Aldrich Chemical Co. It was not reported if the test substance was analytically verified though it was noted that one of three techniques was used to analyze toxicants in water samples from fish exposure tanks.
	Metric 3: Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	The response of the negative control was reported and was adequate for the outcome of interest.
	Metric 6: Randomized Allocation	Medium	It was reported that fish were removed from a common pool and distributed at random among exposure chambers.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test chemicals were prepared using a variety of methods: diluted stock, emulsified stock, liquid-liquid equilibrator, and glass wool equilibrator depending on the test substance characteristic. An 84mg/L stock was prepared by diluting stock where blending the test substance vigorously was the mixing method. A flow-through system was reported using one of three systems: a continuous-flow modified min-diluter, an ABC solenoid operated electronic diluter, or a single-cell glass column system. The exact system was not reported for o-DCB.
	Metric 8: Consistency of Exposure Administration	Medium	Several test chemicals were assessed for this reference. There were slight minor variations in how the tests were conducted based on the year the tests were conducted and the location testing occurred (not all tests were conducted in the same laboratory). Fish were either cultured at U.S. EPA or at the University of Wisconsin-Superior depending on the chemical tested. These were minor conditions that likely would not greatly affect the outcome of the studies. Testing for o-DCB itself and its administration appeared consistent across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Gas-liquid chromatography was used to measure test concentrations. Tests were sampled at 0, 24, 48, 72, and 96h, and all samples were analyzed immediately or preserved for later analysis.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five test concentration, and spacing was adequate to see a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	All test concentrations were well below the water solubility limit.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The fathead minnows were reported to be 32d of age. All test fish were cultured at the U.S. EPA Environmental Research Laboratory in Duluth, MN and at the University of Wisconsin-Superior campus.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It was reported that embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Culture temperature and photoperiod were also the same as test conditions.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 20 fish per test treatment, but the number of replicates was unclear.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Temperature, D.O., hardness, alkalinity, and pH were all reported and monitored. Culture conditions were similar to test conditions, and fish were fed brine shrimp during culturing. A 16L:8D photoperiod was maintained during culturing and for the duration of the test period.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The number of dead fish was noted every 24h after the beginning of the test. Changes in behavior and signs of toxicity were noted at 2-8, 24, 48, 72, and 96h.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Culture conditions and test conditions were described in detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate for the outcomes of interest.
	Metric 22: Reporting of Data	High	Data for o-DCB outcomes was reported in the document 111/112 (p130/347 in the PDF). Data for the control response and the exposure response were reported here along with the estimated 96h LC50 value. Response curves were also reported here.
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Study Citation: Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): Volume III. :328.
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*; Juvenile
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 32170; Linked HERO ID(s): 32170, 3660853

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Confidence limits for the LC50 value were reported.

Additional Comments: This evaluation is for the acute toxicity of o-DCB on juvenile fathead minnows. Mortality was assessed and a 96h LC50 value was reported.

Overall Quality Determination High

Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN.
	Metric 2: Test Substance Source	Low	The o-DCB was reported to be from Aldrich Chemical Co. It was not reported if the test substance was analytically verified though it was noted that one of three techniques was used to analyze toxicants in water samples from fish exposure tanks.
	Metric 3: Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	The response of the negative control was reported and was adequate for the outcome of interest. This was reported as the "effect" outcome.
	Metric 6: Randomized Allocation	Medium	It was reported that fish were removed from a common pool and distributed at random among exposure chambers.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test chemicals were prepared using a variety of methods: diluted stock, emulsified stock, liquid-liquid equilibrator, and glass wool equilibrator depending on the test substance characteristic. An 84mg/L stock was prepared by diluting stock where blending the test substance vigorously was the mixing method. A flow-through system was reported using one of three systems: a continuous-flow modified min-diluter, an ABC solenoid operated electronic diluter, or a single-cell glass column system.
	Metric 8: Consistency of Exposure Administration	Medium	Several test chemicals were assessed for this reference. There were slight minor variations in how the tests were conducted based on the year the tests were conducted and the location testing occurred (not all tests were conducted in the same laboratory). Fish were either cultured at U.S. EPA or at the University of Wisconsin-Superior depending on the chemical tested. These were minor conditions that likely would not greatly affect the outcome of the studies. Testing for o-DCB itself and its administration appeared consistent across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Gas-liquid chromatography was used to measure test concentrations. Tests were sampled at 0, 24, 48, 72, and 96h, and all samples were analyzed immediately or preserved for later analysis.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five test concentration, and spacing was adequate to see a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	All test concentrations were well below the water solubility limit.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The fathead minnows were reported to be 32d of age. All test fish were cultured at the U.S. EPA Environmental Research Laboratory in Duluth, MN and at the University of Wisconsin-Superior campus.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It was reported that embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Culture temperature and photoperiod were also the same as test conditions.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 20 fish per test treatment, but the number of replicates was unclear.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Temperature, D.O., hardness, alkalinity, and pH were all reported and monitored. Culture conditions were similar to test conditions, and fish were fed brine shrimp during culturing. A 16L:8D photoperiod was maintained during culturing and for the duration of the test period.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—signs of toxicity including pigmentation changes and deformations.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The number of dead fish was noted every 24h after the beginning of the test. Changes in behavior and signs of toxicity (changes in pigmentation, deformations, etc.) were noted at 2-8, 24, 48, 72, and 96h.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Culture conditions and test conditions were described in detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate for the outcomes of interest.
	Metric 22: Reporting of Data	Medium	Data for o-DCB outcomes was reported in the document 111/112 (p130/347 in the PDF). Data for the control response and the exposure response were reported here along with the estimated 96h EC50 value. Response curves were also reported here. Note that the changes in skin pigmentation are reported as the "effect" outcome. It is not specified how many fish displayed changes in pigmentation, but signs of toxicity were noted in the "Remarks" section.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.
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; <i>Pimephales promelas</i> ; Juvenile
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report any unexpected outcomes. Confidence limits for the EC50 value were not reported.

Additional Comments: This evaluation is for the acute toxicity of o-DCB on juvenile fathead minnows. An EC50 value was reported for changes in behavior (evaluated separately) due to toxicity. Other contributing factors in determining the EC50 include skin pigmentation (evaluated separately) and reported deformation. This evaluation is for the deformation reported in the remarks. Development/growth was selected as the outcome of interest.

Overall Quality Determination **High**

Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Behavioral		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN.
	Metric 2: Test Substance Source	Low	The o-DCB was reported to be from Aldrich Chemical Co. It was not reported if the test substance was analytically verified though it was noted that one of three techniques was used to analyze toxicants in water samples from fish exposure tanks.
	Metric 3: Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	The response of the negative control was reported and was adequate for the outcome of interest.
	Metric 6: Randomized Allocation	Medium	It was reported that fish were removed from a common pool and distributed at random among exposure chambers.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test chemicals were prepared using a variety of methods: diluted stock, emulsified stock, liquid-liquid equilibrator, and glass wool equilibrator depending on the test substance characteristic. An 84mg/L stock was prepared by diluting stock where blending the test substance vigorously was the mixing method. A flow-through system was reported using one of three systems: a continuous-flow modified min-diluter, an ABC solenoid operated electronic diluter, or a single-cell glass column system.
	Metric 8: Consistency of Exposure Administration	Medium	Several test chemicals were assessed for this reference. There were slight minor variations in how the tests were conducted based on the year the tests were conducted and the location testing occurred (not all tests were conducted in the same laboratory). Fish were either cultured at U.S. EPA or at the University of Wisconsin-Superior depending on the chemical tested. These were minor conditions that likely would not greatly affect the outcome of the studies. Testing for o-DCB itself and its administration appeared consistent across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Gas-liquid chromatography was used to measure test concentrations. Tests were sampled at 0, 24, 48, 72, and 96h, and all samples were analyzed immediately or preserved for later analysis.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five test concentration, and spacing was adequate to see a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	All test concentrations were well below the water solubility limit.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Behavioral		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The fathead minnows were reported to be 32d of age. All test fish were cultured at the U.S. EPA Environmental Research Laboratory in Duluth, MN and at the University of Wisconsin-Superior campus.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It was reported that embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Culture temperature and photoperiod were also the same as test conditions.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 20 fish per test treatment, but the number of replicates was unclear.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Temperature, D.O., hardness, alkalinity, and pH were all reported and monitored. Culture conditions were similar to test conditions, and fish were fed brine shrimp during culturing. A 16L:8D photoperiod was maintained during culturing and for the duration of the test period.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—behavioral changes due to toxicity.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The number of dead fish was noted every 24h after the beginning of the test. Changes in behavior and signs of toxicity were noted at 2-8, 24, 48, 72, and 96h.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Culture conditions and test conditions were described in detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate for the outcomes of interest.
	Metric 22: Reporting of Data	High	Data for o-DCB outcomes was reported in the document 111/112 (p130/347 in the PDF). Data for the control response and the exposure response were reported here along with the estimated 96h EC50 value. Response curves were also reported here.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report any unexpected outcomes. Confidence limits for the EC50 value were not reported.

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Study Citation: Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): Volume III. :328.
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*; Juvenile
Health Outcome: Behavioral
Chemical: o-Dichlorobenzene
HERO ID: 32170; Linked HERO ID(s): 32170, 3660853

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the acute toxicity of o-DCB on juvenile fathead minnows. An EC50 value was reported for changes in behavior due to toxicity. Other contributing factors in determining the EC50 include skin pigmentation and reported deformation (both evaluated separately).		

Overall Quality Determination **High**

Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Neurological		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN.
	Metric 2: Test Substance Source	Low	The o-DCB was reported to be from Aldrich Chemical Co. It was not reported if the test substance was analytically verified though it was noted that one of three techniques was used to analyze toxicants in water samples from fish exposure tanks.
	Metric 3: Test Substance Purity	High	The purity was reported as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	The response of the negative control was reported and was adequate for the outcome of interest. This was reported as the "effect" outcome.
	Metric 6: Randomized Allocation	Medium	It was reported that fish were removed from a common pool and distributed at random among exposure chambers.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test chemicals were prepared using a variety of methods: diluted stock, emulsified stock, liquid-liquid equilibrator, and glass wool equilibrator depending on the test substance characteristic. An 84mg/L stock was prepared by diluting stock where blending the test substance vigorously was the mixing method. A flow-through system was reported using one of three systems: a continuous-flow modified min-diluter, an ABC solenoid operated electronic diluter, or a single-cell glass column system.
	Metric 8: Consistency of Exposure Administration	Medium	Several test chemicals were assessed for this reference. There were slight minor variations in how the tests were conducted based on the year the tests were conducted and the location testing occurred (not all tests were conducted in the same laboratory). Fish were either cultured at U.S. EPA or at the University of Wisconsin-Superior depending on the chemical tested. These were minor conditions that likely would not greatly affect the outcome of the studies. Testing for o-DCB itself and its administration appeared consistent across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Gas-liquid chromatography was used to measure test concentrations. Tests were sampled at 0, 24, 48, 72, and 96h, and all samples were analyzed immediately or preserved for later analysis.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 96h, which is appropriate for an acute toxicity test with fish.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five test concentration, and spacing was adequate to see a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	All test concentrations were well below the water solubility limit.

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Study Citation:	Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (<i>Pimephales promelas</i>): Volume III. :328.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	Neurological		
Chemical:	o-Dichlorobenzene		
HERO ID:	32170; Linked HERO ID(s): 32170, 3660853		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The fathead minnows were reported to be 32d of age. All test fish were cultured at the U.S. EPA Environmental Research Laboratory in Duluth, MN and at the University of Wisconsin-Superior campus.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It was reported that embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Culture temperature and photoperiod were also the same as test conditions.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 20 fish per test treatment, but the number of replicates was unclear.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Temperature, D.O., hardness, alkalinity, and pH were all reported and monitored. Culture conditions were similar to test conditions, and fish were fed brine shrimp during culturing. A 16L:8D photoperiod was maintained during culturing and for the duration of the test period.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—signs of toxicity including pigmentation changes, deformations, convulsions, and rigid musculature.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. The number of dead fish was noted every 24h after the beginning of the test. Changes in behavior and signs of toxicity (changes in pigmentation, deformations, etc.) were noted at 2-8, 24, 48, 72, and 96h.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Culture conditions and test conditions were described in detail.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described in the "Data Analysis" section of the paper and were appropriate for the outcomes of interest.

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Study Citation: Geiger, D. L., Poirier, S. H., Brooke, L. T., Call, D. J. (1986). Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): Volume III. :328.
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*; Juvenile
Health Outcome: Neurological
Chemical: o-Dichlorobenzene
HERO ID: 32170; Linked HERO ID(s): 32170, 3660853

Domain	Metric	Rating	Comments
	Metric 22: Reporting of Data	Medium	Data for o-DCB outcomes was reported in the document 111/112 (p130/347 in the PDF). Data for the control response and the exposure response were reported here along with the estimated 96h EC50 value. Response curves were also reported here. Note that the changes in skin pigmentation are reported as the "effect" outcome. It is not specified how many fish displayed changes in pigmentation, but signs of toxicity were noted in the "Remarks" section.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report any unexpected outcomes. Confidence limits for the EC50 value were not reported.

Additional Comments: This evaluation is for the acute toxicity of o-DCB on juvenile fathead minnows. An EC50 value was reported for changes in behavior (evaluated separately) due to toxicity. Other contributing factors in determining the EC50 include neurological outcomes like convulsions and rigid musculature. This evaluation is for the changes neurological outcomes.

Overall Quality Determination

High

Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.		
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; <i>Pimephales promelas</i> ; Juvenile		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	654569		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Medium	The test substance was identified by name. Several physiochemical properties were reported for each chemical in Table 1.
Metric 2:	Test Substance Source	Low	The manufacture source was reported (British Drug Houses, Ltd, Poole England), but the chemical was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	The purity was reported as 95%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Two controls were used for this LC50 bioassay.
Metric 5:	Negative Control Response	Low	No mortalities or abnormal behavior were reported in the control group. Actual results for the control were not presented, only LC50 values and LBB values.
Metric 6:	Randomized Allocation	Low	No details on allocation to test concentrations were provided.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	Preparation methods of the test media for o-DCB were detailed as preparation within an ultrasonic water bath, and measured concentrations were detailed at water renewals.
Metric 8:	Consistency of Exposure Administration	High	This was a semi-static bioassay with twice a day water renewal (chemical concentration tested). The test chamber volumes were reported, and the water quality parameters were measured throughout the 4-day testing period. Criteria for death was detailed. The study also Cites OECD Test No 203 as a guideline. The test compound administration was consistent for twice daily renewals across the 4 day bioassay.
Metric 9:	Measurement of Test Substance Concentration	High	Water was collected twice daily and GC methods were listed for concentration verification. Recovery details for spiked samples was also performed.
Metric 10:	Exposure Duration and Frequency	High	This study followed OECD Guidelines Test No 203 for a 96-hr LC50 bioassay.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The spacing was adequate to create a dose-response curve. Authors did not indicate that a range finding examination was performed.
Metric 12:	Testing at or Below Solubility Limit	High	O-Dichlorobenzene has a reported solubility of 156 mg/L (NLM 2014), and the test concentrations reported in this work are under those limits.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The original source of the fish was not reported, but they were reported as "raised in our laboratory."
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Details on acclimatization were not reported.

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Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.			
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; <i>Pimephales promelas</i> ; Juvenile			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	654569			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms was reported per concentration and authors cited the use of OECD test guidelines #203. The number of replicates was not specifically stated in this study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	PH, dissolved oxygen, and temperature were recorded three times a day with water exchanges performed twice a day. The biomass loading for this work appears to be 0.9 g/L. EPA series 850 guidelines for biomass loading is 0.8 g/L. Little information was reported on culturing conditions of the fish including feeding regimen.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methods and timing were appropriate for the creation of LC50 and LBB (Lethal Body Burden).	
	Metric 18: Consistency of Outcome Assessment	High	Consistency was present across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Temperature and pH were reported to not vary across the study, however, authors reported that dissolved oxygen decreased to 20% of saturation in the LC50 study and 50% of saturation in the LBB experiments.	
	Metric 20: Outcomes Unrelated to Exposure	High	The control and lower concentration treatments were reported to have no mortalities, and authors stated that "Fish most probably did not die as a result of the low oxygen concentration per se because no mortalities were observed in the control groups experiencing similar deviations." Mean DO concentrations and SDs are presented in Table 2.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical analysis for estimations of LC50s and LBB were detailed in the methods.	
	Metric 22: Reporting of Data	Low	Authors presented the estimated LC50s and LBB values with 95% confidence intervals only, not by individual treatment level and control.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were encountered.	
Additional Comments:	This study determined the lethal body burden for <i>Pimephales promelas</i> for o-Dichlorobenzene. The authors reported many important quality aspects of the study, including chemical source/purity, treatment concentration validation via GC, and environmental parameters. Authors reported following OECD guidelines #203. This OECD guideline details that (20, page 7) "a minimum of 7 fish must be used at each test concentration and control. The fish should be randomly distributed among treatments. No test tank replication is required". The current study used 10 fish per treatment and replication was not stated.			

Overall Quality Determination

High

Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.
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; <i>Pimephales promelas</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	654569

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Medium	The test substance was identified by name. Several physiochemical properties were reported for each chemical in Table 1.
Metric 2:	Test Substance Source	Low	The manufacture source was reported (British Drug Houses, Ltd, Poole England), but the chemical was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	The purity was reported as 95%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Two controls were used for this LC50 bioassay.
Metric 5:	Negative Control Response	Low	No mortalities or abnormal behavior were reported in the control group. Actual results for the control were not presented, only LC50 values and LBB values.
Metric 6:	Randomized Allocation	Low	No details on allocation to test concentrations were provided.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	Preparation methods of the test media for o-DCB were detailed as preparation within an ultrasonic water bath, and measured concentrations were detailed at water renewals.
Metric 8:	Consistency of Exposure Administration	High	This was a semi-static bioassay with twice a day water renewal (chemical concentration tested). The test chamber volumes were reported, and the water quality parameters were measured throughout the 4-day testing period. Criteria for death was detailed. The study also Cites OECD Test No 203 as a guideline. The test compound administration was consistent for twice daily renewals across the 4 day bioassay.
Metric 9:	Measurement of Test Substance Concentration	High	Water was collected twice daily and GC methods were listed for concentration verification. Recovery details for spiked samples was also performed.
Metric 10:	Exposure Duration and Frequency	High	This study followed OECD Guidelines Test No 203 for a 96-hr LC50 bioassay.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The spacing was adequate to create a dose-response curve. Authors did not indicate that a range finding examination was performed.
Metric 12:	Testing at or Below Solubility Limit	High	o-Dichlorobenzene has a reported solubility of 156 mg/L (NLM 2014), and the test concentrations reported in this work are under those limits.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The original source of the fish was not reported, but they were reported as "raised in our laboratory."
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Details on acclimatization were not reported.

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Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.			
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; <i>Pimephales promelas</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	654569			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms was reported per concentration and authors cited the use of OECD test guidelines #203. The number of replicates was not specifically stated in this study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	PH, dissolved oxygen, and temperature were recorded three times a day with water exchanges performed twice a day. The biomass loading for this work appears to be 0.9 g/L. EPA series 850 guidelines for biomass loading is 0.8 g/L. Little information was reported on culturing conditions of the fish including feeding regimen.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methods and timing were appropriate for the creation of LC50 and LBB (Lethal Body Burden).	
	Metric 18: Consistency of Outcome Assessment	High	Consistency was present across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Temperature and pH were reported to not vary across the study, however, authors reported that dissolved oxygen decreased to 20% of saturation in the LC50 study and 50% of saturation in the LBB experiments.	
	Metric 20: Outcomes Unrelated to Exposure	High	The control and lower concentration treatments were reported to have no mortalities, and authors stated that "Fish most probably did not die as a result of the low oxygen concentration per se because no mortalities were observed in the control groups experiencing similar deviations." Mean DO concentrations and SDs are presented in Table 2.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical analysis for estimations of LC50s and LBB were detailed in the methods.	
	Metric 22: Reporting of Data	Low	Authors presented the estimated LC50s and LBB values with 95% confidence intervals only, not by individual treatment level and control.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were encountered.	
Additional Comments:	This study determined the LC50 and lethal body burden for <i>Poecilia reticulata</i> for o-Dichlorobenzene. The authors reported many important quality aspects of the study, including chemical source/purity, treatment concentration validation via GC, and environmental parameters. Authors reported following OECD guidelines #203. This OECD guideline details that (20, page 7) "a minimum of 7 fish must be used at each test concentration and control. The fish should be randomly distributed among treatments. No test tank replication is required". The current study used 10 fish per treatment and replication was not stated.			

Overall Quality Determination**High**

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Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.
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; <i>Pimephales promelas</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	654569

Domain	Metric	Rating	Comments
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Study Citation:	Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. <i>Ecotoxicology and Environmental Safety</i> 35(3):236.			
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; <i>Pimephales promelas</i> ; Adult			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	5253177			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.	
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Purity of the test substance was reported as >95%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	A concurrent negative control group was not included or reported.	
	Metric 5: Negative Control Response	Uninformative	No control was used or reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but did not completely account for physical-chemical properties.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using a gas chromatograph.	
	Metric 10: Exposure Duration and Frequency	Medium	Authors reported the experiment lasted 20 hours, but it is unclear why it was terminated at that point (i.e. all fish died or that was the original plan).	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure concentration used.	
	Metric 12: Testing at or Below Solubility Limit	Low	There was no mention of using a solvent. Authors mentioned adding the test chemical to the water by dissolving the chemical at approximately 50% of the aqueous solubility and stirring for one day. More than 80% of the test chemical remained in the aquarium at test termination so there likely was not an issue with solubility, but still there is uncertainty surrounding this due to lack of details presented.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for a week prior to test start.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were ten fish placed in the aquarium, but there was only one tank used for just o-dichlorobenzene.	

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Study Citation:	Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. <i>Ecotoxicology and Environmental Safety</i> 35(3):236.
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; <i>Pimephales promelas</i> ; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5253177

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	There were few details given on environmental conditions during the test.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome with minor uncertainties.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Details on environmental conditions were limited.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was reported in the paper but there was only one aquarium for o-dichlorobenzene so statistical analysis was not appropriate.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented for aquarium F in Table 2 and lethal body burden for the fathead minnow in this study in Table 3, but no control data was shown. No raw data was shown either.
	Metric 23: Explanation of Unexpected Outcomes	High	Standard deviation was shown in Table 2 and Table 3.

Additional Comments: All but one exposure level was a mixture for this test.

Overall Quality Determination

Uninformative

Study Citation:	Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. <i>Ecotoxicology and Environmental Safety</i> 35(3):236.
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; <i>Pimephales promelas</i> ; Adult
Health Outcome:	Behavioral
Chemical:	o-Dichlorobenzene
HERO ID:	5253177

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	Purity of the test substance was reported as >95%.
Domain 2: Test Design			
	Metric 4: Negative Controls	Uninformative	A concurrent negative control group was not included or reported.
	Metric 5: Negative Control Response	Uninformative	No control was used or reported.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but did not completely account for physical-chemical properties.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using a gas chromatograph.
	Metric 10: Exposure Duration and Frequency	Medium	Authors reported the experiment lasted 20 hours, but it is unclear why it was terminated at that point (i.e. all fish died or that was the original plan).
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure concentration used.
	Metric 12: Testing at or Below Solubility Limit	Low	There was no mention of using a solvent. Authors mentioned adding the test chemical to the water by dissolving the chemical at approximately 50% of the aqueous solubility and stirring for one day. More than 80% of the test chemical remained in the aquarium at test termination so there likely was not an issue with solubility, but still there is uncertainty surrounding this due to lack of details presented.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described.
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for a week prior to test start.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were ten fish placed in the aquarium, but there was only one tank used for just o-dichlorobenzene.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	There were few details given on environmental conditions during the test.

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Study Citation: Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. *Ecotoxicology and Environmental Safety* 35(3):236.
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*; Adult
Health Outcome: Behavioral
Chemical: o-Dichlorobenzene
HERO ID: 5253177

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome with minor uncertainties.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Details on environmental conditions were limited.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was reported in the paper but there was only one aquarium for o-dichlorobenzene so statistical analysis was not appropriate.
	Metric 22: Reporting of Data	Low	General behavior results were mentioned in the text under 'Sublethal Effects', but nothing was specifically described for aquarium A. Nothing was mentioned regarding control behavior.
	Metric 23: Explanation of Unexpected Outcomes	Low	No quantitative behavior data was shown and no measures of variability provided. Results were vaguely described.

Additional Comments: All but one exposure level was a mixture for this test.

Overall Quality Determination

Uninformative

Study Citation:	Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. <i>Ecotoxicology and Environmental Safety</i> 35(3):236.
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; <i>Pimephales promelas</i> ; Adult
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5253177

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.
	Metric 2: Test Substance Source	Low	The test substance identity was not analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	Purity of the test substance was reported as >95%.
Domain 2: Test Design			
	Metric 4: Negative Controls	Uninformative	A concurrent negative control group was not included or reported.
	Metric 5: Negative Control Response	Uninformative	No control was used or reported.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and/or test media preparation methods were adequately reported but did not completely account for physical-chemical properties.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured using a gas chromatograph.
	Metric 10: Exposure Duration and Frequency	Medium	Authors reported the experiment lasted 20 hours, but it is unclear why it was terminated at that point (i.e. all fish died or that was the original plan).
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure concentration used.
	Metric 12: Testing at or Below Solubility Limit	Low	There was no mention of using a solvent. Authors mentioned adding the test chemical to the water by dissolving the chemical at approximately 50% of the aqueous solubility and stirring for one day. More than 80% of the test chemical remained in the aquarium at test termination so there likely was not an issue with solubility, but still there is uncertainty surrounding this due to lack of details presented.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described.
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions for a week prior to test start.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were ten fish placed in the aquarium, but there was only one tank used for just o-dichlorobenzene.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	There were few details given on environmental conditions during the test.

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Study Citation: Wezel, Van, A. P., Vries, De, M., D.A., Sijm, H.,M, D.T., Opperhuizen, A. (1996). Use of lethal body burden in the evaluation of mixture toxicity. *Ecotoxicology and Environmental Safety* 35(3):236.
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*; Adult
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 5253177

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome with minor uncertainties.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Details on environmental conditions were limited.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was reported in the paper but there was only one aquarium for o-dichlorobenzene so statistical analysis was not appropriate.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were presented for aquarium A in Table 2, but no control data was shown. No raw data for mortality was shown.
	Metric 23: Explanation of Unexpected Outcomes	High	Standard deviation was shown in Table 2.

Additional Comments: All but one exposure level was a mixture for this test.

Overall Quality Determination

Uninformative

Study Citation:	Hall, L., Kier, L., Phipps, G. (1984). STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON THE TOXICITIES OF BENZENE-DERIVATIVES .1. AN ADDITIVITY MODEL. Environmental Toxicology and Chemistry 3(3):355-365.			
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	11455129			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the o-DCB was not reported.	
	Metric 3: Test Substance Purity	Low	The purity of the o-DCB was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported. Only log(LC50) values were reported.	
	Metric 6: Randomized Allocation	Medium	It was reported that 50 fish were randomly assigned to 12 exposure tanks in each flow-through test. In the case of static tests, 20 fish were placed in each exposure chamber.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The test system was reported to be a flow-through system that used cycle proportional diluters with a dilution factor of 0.6. Diluters were reported to cycle at a rate of 10 tank volumes a day. It was also reported that static systems were used, and it is unclear which test substances used which system. The method of preparation of the test substance was not reported in detail.	
	Metric 8: Consistency of Exposure Administration	Low	All tests were conducted using two comparable 2L/cycles proportional diluters with a dilution factor of 0.6. Glass aquaria that were 30cm x 30cm x 60cm were used in all tests. Water volume was 41L. A 16L photoperiod was also used in all tests. Static tests used stainless steel and glass aquaria that were 23cm x 23cm x 17cm with a water volume of 9L. It was unclear which test system was used for each test chemical.	
	Metric 9: Measurement of Test Substance Concentration	Low	Some of the test substance were reported to be measured analytically, but it is unclear if all of them were analyzed. Measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	Low	The abstract indicates that 96h toxicity values were used in the QSAR. However, it appears that the tests were run for eight days in the methods, which is longer than is typical. It is unclear if LC50 values were determined at several time points within the study duration or not.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported that there were five exposure groups with a factor of 0.6. However, the actual test concentrations were not reported.	
	Metric 12: Testing at or Below Solubility Limit	Low	Actual test concentrations were not reported, so it is unclear if any were above the water solubility limit.	
Domain 4: Test Organism				

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Study Citation:	Hall, L., Kier, L., Phipps, G. (1984). STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON THE TOXICITIES OF BENZENE-DERIVATIVES .1. AN ADDITIVITY MODEL. Environmental Toxicology and Chemistry 3(3):355-365.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	11455129

Domain	Metric	Rating	Comments
	Metric 13: Test Organism Characteristics	Low	The source of the fathead minnows was not reported. It was reported that they were laboratory reared, but the actual source was not explicitly stated. It was reported that the fish were 30-35d old in all experiments.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It was reported that fish were reared in a system similar to the exposure systems at similar temperatures with similar diluent water.
	Metric 15: Number of Organisms and Replicates per Group	Medium	In the flow-through tests, 50 fish were assigned to each exposure tank. In the static tests, 20 fish were assigned to each exposure tank. Each exposure was conducted in duplicate.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The exposure system was reported to use Lake Superior water at 25C. DO, hardness, alkalinity, and pH were determined at the beginning, middle and the end of each 8-d test. Rearing conditions were reported to be similar to test conditions in similar water at the same temperature. They were fed brine shrimp until 12-24h prior to testing and were not fed for the duration of the test.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality in terms of logLC50 values.
	Metric 18: Consistency of Outcome Assessment	High	It was reported that dead fish were counted and removed four times during the first day and twice daily after that point.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment. Rearing conditions and test conditions were reported to be similar.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	LC50 values were determined to be calculated using the Trimmed Spearman-Kärber Method.
	Metric 22: Reporting of Data	Low	Only log(LC50) values were reported. Data for each test concentration and the control response were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report any measures of variability. Confidence intervals were not reported.

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Study Citation:	Hall, L., Kier, L., Phipps, G. (1984). STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON THE TOXICITIES OF BENZENE-DERIVATIVES .1. AN ADDITIVITY MODEL. Environmental Toxicology and Chemistry 3(3):355-365.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; <i>Pimephales promelas</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	11455129

Domain	Metric	Rating	Comments
Additional Comments:	This study reported log LC50 values for several test chemicals in order to perform a quantitative structure-activity analysis. This evaluation is for the mortality outcome of fathead minnows exposed to o-DCB. A value for o-DCB was also reported, but this was taken from another reference. The decision was made to extract the o-DCB data because the source of the data point (Koneman, H. 1979) appeared to be a dissertation not available as a PDF.		

Overall Quality Determination

Medium

Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.		
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; <i>Poecilia reticulata</i> ; Juvenile		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	654569		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Medium	The test substance was identified by name. Several physiochemical properties were reported for each chemical in Table 1.
Metric 2:	Test Substance Source	Low	The manufacture source was reported (British Drug Houses, Ltd, Poole England), but the chemical was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	The purity was reported as 95%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Two controls were used for this LC50 bioassay.
Metric 5:	Negative Control Response	Low	No mortalities or abnormal behavior were reported in the control group. Actual results for the control were not presented, only LC50 values and LBB values.
Metric 6:	Randomized Allocation	Low	No details on allocation to test concentrations were provided.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	Preparation methods of the test media for o-DCB were detailed as preparation within an ultrasonic water bath, and measured concentrations were detailed at water renewals.
Metric 8:	Consistency of Exposure Administration	High	This was a semi-static bioassay with twice a day water renewal (chemical concentration tested). The test chamber volumes were reported, and the water quality parameters were measured throughout the 4-day testing period. Criteria for death was detailed. The study also Cites OECD Test No 203 as a guideline. The test compound administration was consistent for twice daily renewals across the 4 day bioassay.
Metric 9:	Measurement of Test Substance Concentration	High	Water was collected twice daily and GC methods were listed for concentration verification. Recovery details for spiked samples was also performed.
Metric 10:	Exposure Duration and Frequency	High	This study followed OECD Guidelines Test No 203 for a 96-hr LC50 bioassay.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The spacing was adequate to create a dose-response curve. Authors did not indicate that a range finding examination was performed.
Metric 12:	Testing at or Below Solubility Limit	High	O-Dichlorobenzene has a reported solubility of 156 mg/L (NLM 2014), and the test concentrations reported in this work are under those limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The original source of the fish was not reported, but they were reported as "raised in our laboratory."
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Details on acclimatization were not reported.

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Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.			
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; <i>Poecilia reticulata</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	654569			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms was reported per concentration and authors cited the use of OECD test guidelines #203. The number of replicates was not specifically stated in this study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	PH, dissolved oxygen, and temperature were recorded three times a day with water exchanges performed twice a day. The biomass loading for this work appears to be 0.9 g/L. EPA series 850 guidelines for biomass loading is 0.8 g/L. Little information was reported on culturing conditions of the fish including feeding regimen.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methods and timing were appropriate for the creation of LC50 and LBB (Lethal Body Burden).	
	Metric 18: Consistency of Outcome Assessment	High	Consistency was present across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Temperature and pH were reported to not vary across the study, however, authors reported that dissolved oxygen decreased to 20% of saturation in the LC50 study and 50% of saturation in the LBB experiments.	
	Metric 20: Outcomes Unrelated to Exposure	High	The control and lower concentration treatments were reported to have no mortalities, and authors stated that "Fish most probably did not die as a result of the low oxygen concentration per se because no mortalities were observed in the control groups experiencing similar deviations." Mean DO concentrations and SDs are presented in Table 2.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical analysis for estimations of LC50s and LBB were detailed in the methods.	
	Metric 22: Reporting of Data	Low	Authors presented the estimated LC50s and LBB values with 95% confidence intervals only, not by individual treatment level and control.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were encountered.	
Additional Comments:	This study determined the LC50 for <i>Poecilia reticulata</i> for o-Dichlorobenzene. The authors reported many important quality aspects of the study, including chemical source/purity, treatment concentration validation via GC, and environmental parameters. Authors reported following OECD guidelines #203. This OECD guideline details that (20, page 7) "a minimum of 7 fish must be used at each test concentration and controls. The fish should be randomly distributed among treatments. No test tank replication is required". The current study used 10 fish per treatment and replication was not stated.			

Overall Quality Determination**High**

Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.		
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; <i>Poecilia reticulata</i> ; Juvenile		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	654569		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Medium	The test substance was identified by name. Several physiochemical properties were reported for each chemical in Table 1.
Metric 2:	Test Substance Source	Low	The manufacture source was reported (British Drug Houses, Ltd, Poole England), but the chemical was not analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	The purity was reported as 95%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Two controls were used for this LC50 bioassay.
Metric 5:	Negative Control Response	Low	No mortalities or abnormal behavior were reported in the control group. Actual results for the control were not presented, only LC50 values and LBB values.
Metric 6:	Randomized Allocation	Low	No details on allocation to test concentrations were provided.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	Preparation methods of the test media for o-DCB were detailed as preparation within an ultrasonic water bath, and measured concentrations were detailed at water renewals.
Metric 8:	Consistency of Exposure Administration	High	This was a semi-static bioassay with twice a day water renewal (chemical concentration tested). The test chamber volumes were reported, and the water quality parameters were measured throughout the 4-day testing period. Criteria for death was detailed. The study also Cites OECD Test No 203 as a guideline. The test compound administration was consistent for twice daily renewals across the 4 day bioassay.
Metric 9:	Measurement of Test Substance Concentration	High	Water was collected twice daily and GC methods were listed for concentration verification. Recovery details for spiked samples was also performed.
Metric 10:	Exposure Duration and Frequency	High	This study followed OECD Guidelines Test No 203 for a 96-hr LC50 bioassay.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The spacing was adequate to create a dose-response curve. Authors did not indicate that a range finding examination was performed.
Metric 12:	Testing at or Below Solubility Limit	High	O-Dichlorobenzene has a reported solubility of 156 mg/L (NLM 2014), and the test concentrations reported in this work are under those limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Medium	The original source of the fish was not reported, but they were reported as "raised in our laboratory."
Metric 14:	Acclimatization and Pretreatment Conditions	Low	Details on acclimatization were not reported.

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Study Citation:	Sum, H.,M, D.T., Schipper, M., Opperhuizen, A. (1993). Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environmental Toxicology and Chemistry 12(6):1117-1127.			
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; <i>Poecilia reticulata</i> ; Juvenile			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	654569			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of test organisms was reported per concentration and authors cited the use of OECD test guidelines #203. The number of replicates was not specifically stated in this study.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	PH, dissolved oxygen, and temperature were recorded three times a day with water exchanges performed twice a day. The biomass loading for this work appears to be 0.9 g/L. EPA series 850 guidelines for biomass loading is 0.8 g/L. Little information was reported on culturing conditions of the fish including feeding regimen.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methods and timing were appropriate for the creation of LC50 and LBB (Lethal Body Burden).	
	Metric 18: Consistency of Outcome Assessment	High	Consistency was present across study groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Temperature and pH were reported to not vary across the study, however, authors reported that dissolved oxygen decreased to 20% of saturation in the LC50 study and 50% of saturation in the LBB experiments.	
	Metric 20: Outcomes Unrelated to Exposure	High	The control and lower concentration treatments were reported to have no mortalities, and authors stated that "Fish most probably did not die as a result of the low oxygen concentration per se because no mortalities were observed in the control groups experiencing similar deviations." Mean DO concentrations and SDs are presented in Table 2.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	The statistical analysis for estimations of LC50s and LBB were detailed in the methods.	
	Metric 22: Reporting of Data	Low	Authors presented the estimated LC50s and LBB values with 95% confidence intervals only, not by individual treatment level and control.	
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were encountered.	
Additional Comments:	This study determined the LBB for <i>Poecilia reticulata</i> for o-Dichlorobenzene. The authors reported many important quality aspects of the study, including chemical source/purity, treatment concentration validation via GC, and environmental parameters. Authors reported following OECD guidelines #203. This OECD guideline details that (20, page 7) "a minimum of 7 fish must be used at each test concentration and controls. The fish should be randomly distributed among treatments. No test tank replication is required". The current study used 10 fish per treatment and replication was not stated.			

Overall Quality Determination**High**

Study Citation:	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vertebrate; Amphibian; <i>Rana pipiens</i> ; Embryo		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	93660		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The chemical source was not reported.
	Metric 3: Test Substance Purity	High	Authors reported that the test substance was reagent grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls were used.
	Metric 5: Negative Control Response	Low	Mortality of the control group was not reported.
	Metric 6: Randomized Allocation	Low	Randomized allocation of organisms to study groups was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system used was well described.
	Metric 8: Consistency of Exposure Administration	High	No test system inconsistencies were reported.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured daily, and analytical methods were reported.
	Metric 10: Exposure Duration and Frequency	High	The toxicity tests were conducted using a flow-through system. The test duration was appropriate to determine hatch percentage and survival. The exposure was initiated at fertilization and maintained through four days post-hatching.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Seven test concentrations were used. This was sufficient to determine an outcome response and calculate an LC50.
	Metric 12: Testing at or Below Solubility Limit	High	The highest test concentration for 1,2-dichlorobenzene was well below the solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms used were described in detail. The source of them was reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Methods of pretreatment were not well documented.
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of organisms used was adequate, but it was reported as a range of organisms used per treatment level. Therefore, the exact number used per treatment was not known, and there was no report of using replicates.
Domain 5: Outcome Assessment			

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Study Citation:	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Amphibian; <i>Rana pipiens</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	93660

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Test conditions were suitable and well documented.
	Metric 17: Outcome Assessment Methodology	High	Methods for assessing percent hatched and survival of the organisms was described. Abnormal organisms were treated as mortalities.
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables such as teratogenesis were explained.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information suggesting differences among the study groups in outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The statistical methods used were adequately described.
	Metric 22: Reporting of Data	High	Mortality and hatch percentage were clearly reported.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported. Measures of variability were shown in Tables 7 and 14.

Additional Comments: This evaluation is for the assessment of percent hatched and survival of test organisms. LC50 values were generated.

Overall Quality Determination

High

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.			
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; <i>Salmo gairdneri</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.	
	Metric 3: Test Substance Purity	Low	The percent purity was not reported. All test chemicals were reported to be of analytical grade.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Limited details were given in the paper with respect to a control. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group for the test substance was not reported.	
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study reported the use of two closed glass bottles for each concentration, but major details regarding test media preparation were not given in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration details are not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured at the beginning and the end of the short-term tests, but they were not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 10: Exposure Duration and Frequency	Low	This was an acute toxicity test conducted for 48 h. According to OECD guidelines for Test No. 203, acute fish toxicity tests should be conducted for 96 h.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups and spacing of exposure levels in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.	
	Metric 12: Testing at or Below Solubility Limit	Low	No details were provided to determine whether exposure concentrations exceeded the water solubility limit.	

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Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.
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; <i>Salmo gairdneri</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the organisms was not reported, but the test organism was appropriate for evaluating the outcome. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 5 fish per concentration and 2 replicates for each concentration. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions during the test were reported, but it could not be determined whether differences occurred between control and exposed populations. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 18:	Consistency of Outcome Assessment	Low	No details were provided regarding the execution of the study protocol for outcome assessment.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information presented to suggest differences among treatment groups were due to exposure unrelated reasons.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described well.
Metric 22:	Reporting of Data	Low	Mortality data for treatments groups and controls were not reported. Only 48h LC 50 values were reported.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes

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Study Citation: Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12(2):253-262.
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; *Salmo gairdneri*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Additional Comments:	The source of test organisms, exposure concentrations (nominal or measured), spacing of exposure levels, and control response were not reported. Only 48 h LC 50 values along with confidence intervals were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	3634370; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the 1,2-DCB was reported to be Aldrich Chemical Co. It was not reported if it was analytically verified.
	Metric 3: Test Substance Purity	High	The purity of the 1,2-DCB was reported to be 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a negative control.
	Metric 5: Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported (Table 2).
	Metric 6: Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The test system was reported to be a flow-through system that used a diluter. Little information was provided on the preparation of the test concentrations.
	Metric 8: Consistency of Exposure Administration	Low	Little information was provided on the exposure administration. There were 10 fish per test chamber with a test temperature of 12C. Test chamber size was uncertain.
	Metric 9: Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using gas-liquid chromatography, the parameters of which are presented in Table C-2. Measured concentrations were reported in Table B-1.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 144h, and a 96h LC50 value was reported.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five reported exposure concentrations (see Table B-1), and the spacing appears adequate for a response.
	Metric 12: Testing at or Below Solubility Limit	High	Measured test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The rainbow trout were reported to be fingerlings from Lake Mills, WI National Fish Hatchery and from Fattig Hatchery of Brady, NE.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	The fish were reported to be acclimated in Lake Superior water at 12C until use in testing, but the acclimation duration was not reported.
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test chamber, but the number of replicates was unclear.

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Study Citation:	Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; <i>Salmo gairdneri</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	3634370; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Test conditions were reported in Table A-1, but little information on culture/holding conditions and feeding regimen were reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality as reported in LC50 values.
	Metric 18: Consistency of Outcome Assessment	High	Mortality was determined every 24h and appeared to be assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Trimmed Spearman-Karber method was reported to be used to determine LC50 values.
	Metric 22: Reporting of Data	Low	Data for each test concentration was not reported. Only LC50 values were reported. Table 2 contains LC50 values for rainbow trout.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. 95% confidence intervals were reported in Table 2.

Additional Comments: This evaluation is for the acute toxicity of o-DCB to rainbow trout. Results are reported in Table 2. The acute toxicity of p-DCB to rainbow trout was not evaluated for this reference. It is evaluated under HERO ID 7494661.

Overall Quality Determination

Medium

Study Citation:	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.		
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; <i>Salmo gairdneri</i> ; Embryo		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	93660		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The chemical source was not reported.
	Metric 3: Test Substance Purity	High	Authors reported that the test substance was reagent grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls were used.
	Metric 5: Negative Control Response	Low	Mortality of the control group was not reported.
	Metric 6: Randomized Allocation	Low	Randomized allocation of organisms to study groups was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system used was well described.
	Metric 8: Consistency of Exposure Administration	High	No test system inconsistencies were reported.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured daily, and analytical methods were reported.
	Metric 10: Exposure Duration and Frequency	High	The toxicity tests were conducted using a flow-through system. The test duration was appropriate to determine hatch percentage and survival. The exposure was initiated at fertilization and maintained through four days post-hatching.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Five test concentrations were used. This was sufficient to determine an outcome response and calculate an LC50.
	Metric 12: Testing at or Below Solubility Limit	High	The highest test concentration for 1,2-dichlorobenzene was well below the solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms used were described in detail. The source of them was reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Methods of pretreatment were not well documented.
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of organisms used was adequate, but it was reported as a range of organisms used per treatment level. Therefore, the exact number used per treatment was not known, and there was no report of using replicates.
Domain 5: Outcome Assessment			

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Study Citation:	Black, J. A., Birge, W. J., McDonnell, W. E., Westerman, A. G., Ramey, B. A., Bruser, D. M. (1982). The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. :61.
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; <i>Salmo gairdneri</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	93660

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Test conditions were suitable and well documented.
	Metric 17: Outcome Assessment Methodology	High	Methods for assessing percent hatched and survival of the organisms was described. Abnormal organisms were treated as mortalities.
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables such as teratogenesis were explained.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information suggesting differences among the study groups in outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The statistical methods used were adequately described.
	Metric 22: Reporting of Data	High	Mortality and hatch percentage were clearly reported.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported. Measures of variability were shown in Tables 7 and 14.

Additional Comments: This evaluation is for the assessment of percent hatched and survival of test organisms. LC50 values were generated.

Overall Quality Determination

High

Study Citation:	Dow Chemical, (1974). Dynamic acute toxicity of selected phenol and benzene compounds to rainbow trout, <i>Salmo gairdneria</i> , Richardson prepared by Waste Control Dept.			
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; <i>Salmo gairdneria</i> ; Juvenile			
Health Outcome:	Neurological			
Chemical:	o-Dichlorobenzene			
HERO ID:	2048390			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was only identified by name.	
	Metric 2: Test Substance Source	Low	The source of o-dichlorobenzene was identified as Fisher Scientific Co. It was not mentioned if the test substance was analytically verified.	
	Metric 3: Test Substance Purity	Low	Purity was reported as practical grade.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls and acetone controls were included in the study.	
	Metric 5: Negative Control Response	High	Negative controls were not reported to have effects.	
	Metric 6: Randomized Allocation	Low	Allocation of organisms was not described in the study.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Temperature, DO, and pH of the system were monitored. Details were vague regarding test media preparation.	
	Metric 8: Consistency of Exposure Administration	High	Exposures were reported consistently.	
	Metric 9: Measurement of Test Substance Concentration	High	The test concentrations were measured at the end of the exposure.	
	Metric 10: Exposure Duration and Frequency	High	The 96-hour duration was appropriate for this acute toxicity exposure.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	Study authors reported using ten test concentrations. Mortality and LC values were determined from the concentrations used.	
	Metric 12: Testing at or Below Solubility Limit	High	The o-dichlorobenzene was diluted in acetone.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source and age of the fish was not specified. The fish used were 2-3 inches long.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported whether test organisms were acclimated prior to test start.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were ten fish per test concentration. The number of replicates used was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Differences were not reported. Test conditions seemed adequate.	
	Metric 17: Outcome Assessment Methodology	High	Intended outcomes were clearly reported.	
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Study Citation: Dow Chemical, (1974). Dynamic acute toxicity of selected phenol and benzene compounds to rainbow trout, *Salmo gairdneria*, Richardson prepared by Waste Control Dept.
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; *Salmo gairdneria*; Juvenile
Health Outcome: Neurological
Chemical: o-Dichlorobenzene
HERO ID: 2048390

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Distress was assessed consistently among all treatment groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences or variables within the test concentrations.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences among test organisms that could influence the mortality assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods used were described in adequate detail on page 8 of the pdf.
	Metric 22: Reporting of Data	Low	Table 2 shows the toxic effect concentrations. In Table 4 there are no EC values for o-Dichlorobenzene shown. Raw distress (defined as loss of balance control) data is not shown.
	Metric 23: Explanation of Unexpected Outcomes	Low	No uncertainties or unexpected outcomes were reported. No measures of variability were shown for o-Dichlorobenzene.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Dow Chemical, (1974). Dynamic acute toxicity of selected phenol and benzene compounds to rainbow trout, <i>Salmo gairdneria</i> , Richardson prepared by Waste Control Dept.		
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; <i>Salmo gairdneria</i> ; Juvenile		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	2048390		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was only identified by name.
	Metric 2: Test Substance Source	Low	The source of o-dichlorobenzene was identified as Fisher Scientific Co. It was not mentioned if the test substance was analytically verified.
	Metric 3: Test Substance Purity	Low	Purity was reported as practical grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls and acetone controls were included in the study.
	Metric 5: Negative Control Response	High	Negative controls were not reported to have effects.
	Metric 6: Randomized Allocation	Low	Allocation of organisms was not described in the study.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Temperature, DO, and pH of the system were monitored. Details were vague regarding test media preparation.
	Metric 8: Consistency of Exposure Administration	High	Exposures were reported consistently.
	Metric 9: Measurement of Test Substance Concentration	High	The test concentrations were measured at the end of the exposure.
	Metric 10: Exposure Duration and Frequency	High	The 96-hour duration was appropriate for this acute toxicity exposure.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Study authors reported using ten test concentrations. Mortality and LC values were determined from the concentrations used.
	Metric 12: Testing at or Below Solubility Limit	High	The o-dichlorobenzene was diluted in acetone.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source and age of the fish was not specified. The fish used were 2-3 inches long.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported whether test organisms were acclimated prior to test start.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were ten fish per test concentration. The number of replicates used was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Differences were not reported. Test conditions seemed adequate.
	Metric 17: Outcome Assessment Methodology	High	Intended outcomes were clearly reported.
	Metric 18: Consistency of Outcome Assessment	High	LC 10, 50, and 90 were reported. Mortality was assessed consistently among all treatment groups.

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Study Citation:	Dow Chemical, (1974). Dynamic acute toxicity of selected phenol and benzene compounds to rainbow trout, <i>Salmo gairdneria</i> , Richardson prepared by Waste Control Dept.
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; <i>Salmo gairdneria</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	2048390

Domain	Metric	Rating	Comments
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences or variables within the test concentrations.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences among test organisms that could influence the mortality assessment.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods used were described in adequate detail on page 8 of the pdf.
Metric 22:	Reporting of Data	Medium	Table 2 shows the 100% fatal and partial kill test concentrations. Table 3 shows the LC values. Raw mortality data is not shown.
Metric 23:	Explanation of Unexpected Outcomes	High	No uncertainties or unexpected outcomes were reported. Measures of variability were included in Table 3.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran <i>Ceriodaphnia cf. dubia</i> . Archives of Environmental Contamination and Toxicology 34(3):248-252.		
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; <i>Ceriodaphnia dubia</i> ; cf Richard; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Immobilization		
Chemical:	o-Dichlorobenzene		
HERO ID:	651551		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by name and CAS #.
Metric 2:	Test Substance Source	Low	The chemical source and any analytical verification were not reported.
Metric 3:	Test Substance Purity	High	All chemicals were >=97 % pure.
Domain 2: Test Design			
Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.
Metric 5:	Negative Control Response	Low	Use of controls was not reported nor was the control response.
Metric 6:	Randomized Allocation	Low	There was no mention of using random allocation.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	Little details were provided on the test system, though headspace was reduced to limit volatilization of the test substance. Preparation of the test concentrations was not described in great detail, but test concentrations were measured and reported to be within 20% of nominal. The concentrations measured were only at test initiation.
Metric 8:	Consistency of Exposure Administration	Low	No inconsistencies were reported, but there was not much information reported either.
Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations were only measured at the beginning of the test.
Metric 10:	Exposure Duration and Frequency	High	Exposure durations followed standard protocol.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of test concentrations and their spacing were not reported. Though US EPA methods were cited, it is possible deviations occurred that were not reported in this paper.
Metric 12:	Testing at or Below Solubility Limit	Medium	EC50 values were well below solubility, acetone was also added to aid in dissolving the chemical.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	Little information was provided the test organisms. The source of the <i>C. dubia</i> was not reported. Warne 1996 was cited for maintenance conditions. Little was reported on test conditions. The age of the organisms was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	No acclimatization conditions were reported.
Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms and replicates per group were not reported.

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Study Citation: Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran *Ceriodaphnia cf. dubia*. Archives of Environmental Contamination and Toxicology 34(3):248-252.

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; *Ceriodaphnia dubia*; cf Richard; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Immobilization

Chemical: o-Dichlorobenzene

HERO ID: 651551

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Test conditions were not reported, only that they followed standard methods. Warne 1996 was cited for maintenance methods.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome of interest-immobilization.
	Metric 18: Consistency of Outcome Assessment	Medium	The outcome assessment method for determining immobilization was not reported in great detail.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods seemed suitable for these determinations.
	Metric 22: Reporting of Data	Low	EC50 values were reported in Table 1. However, data for each concentration group were not reported. Control data was also not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This evaluation is for the *C. cf. dubia* exposure to o-DCB. The EC50 for immobilization was determined after a 48h exposure.

Overall Quality Determination

Uninformative

Study Citation:	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).			
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; <i>Chironomus dilutus</i> ; Larvae			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	11799635			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN in the certificate of analysis provided in Appendix 2.	
Metric 2:	Test Substance Source	High	The test substance was analytically verified as reported in the certificate of analysis provided in Appendix 2.	
Metric 3:	Test Substance Purity	High	The purity of the non-radiolabeled o-DCB was reported to be 99.7%. The radiolabeled test substance was reported to have a radiochemical purity of 97.0 area%.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported the use of both a negative control and a solvent control.	
Metric 5:	Negative Control Response	High	The negative control and solvent control responses were reported in Appendix 9 for the range finding test.	
Metric 6:	Randomized Allocation	Low	It was not reported how organisms were allocated into study groups in the range finding test.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Low	It was reported that the range finding test was conducted under intermittent-renewal systems. Little detail was provided on what this entailed. Preparation of the test sediment was not well described for the range-finder.	
Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the exposure administration in the range-finding test.	
Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured in the range-finding test. Only nominal concentrations were reported.	
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 10 days, which is in accordance with EPA test guidelines for spiked sediment tests.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure concentrations that were spaced quite far apart, but this was appropriate for a range-finding test.	
Metric 12:	Testing at or Below Solubility Limit	N/A	This was a sediment test.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	High	Test organisms were reported to originally be from Aquatic Bio Systems in Fort Collins, CO. A culture was maintained in-house at Eurofins-Easton aquatic ecotoxicology facility. Third-instar larvae were used at test initiation.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated for the range-finding test.	

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Study Citation:	Agroscience., E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (Chironomus dilutus) using spiked whole sediment (o-DCB Test Order).
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; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	11799635

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 midge per test chamber with four replicates per treatment and control group in the range-finding test.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Test conditions for the range-finding test were reported to be similar to the definitive test, but little other information was provided.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—growth in terms of mean ash-free dry weight.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little information was provided on acclimation. It was also only stated that the test conditions for the range-finding test were similar to the definitive test.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical methods for the range-finding test were not described in detail, but could be conducted from data provided in Appendix 9.
	Metric 22: Reporting of Data	High	Growth was reported for all test concentrations and the controls in Appendix 9.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Measures of variability were provided.
Additional Comments:	This evaluation is for a 10d spiked sediment test using o-DCB and <i>C. dilutus</i> larvae. This evaluation was for the range-finding test conducted prior to the definitive test. Ash-free dry weights were taken at the end of the study to determine growth.		

Overall Quality Determination

Medium

Study Citation:	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).
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; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	11799635

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN in the certificate of analysis provided in Appendix 2.
Metric 2:	Test Substance Source	High	The test substance was analytically verified as reported in the certificate of analysis provided in Appendix 2.
Metric 3:	Test Substance Purity	High	The purity of the non-radiolabeled o-DCB was reported to be 99.7%. The radiolabeled test substance was reported to have a radiochemical purity of 97.0 area%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of both a negative control and a solvent control.
Metric 5:	Negative Control Response	High	The negative control and solvent control responses were reported in Table 10 and in Appendix 20 for growth in terms of ash-free dry weight.
Metric 6:	Randomized Allocation	Medium	The midge larvae were reported to be randomly distributed into test groups and controls.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	A flow-through diluter system was used for the test system. Test compartments were 300mL beakers with about 100mL of test or control sediment and 175mL of overlying water. The preparation of the test sediment was reported in adequate detail.
Metric 8:	Consistency of Exposure Administration	High	The exposures were administered consistently across study groups. Test chambers were the same across groups along with the amount of test sediment. Environmental conditions were the same across test groups, and the test substance concentrations were monitored for the duration of the study.
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were analyzed at several time points for the duration of the test (days 0, 5, and 10). Overlying water, pore water, and sediment were all measured at these times. LSC was used to confirm test concentrations.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 10 days, which is in accordance with EPA test guidelines for spiked sediment tests.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure concentrations, which was adequate for a response.
Metric 12:	Testing at or Below Solubility Limit	N/A	This was a sediment test.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Test organisms were reported to originally be from Aquatic Bio Systems in Fort Collins, CO. A culture was maintained in-house at Eurofins-Easton aquatic ecotoxicology facility. Third-instar larvae were used at test initiation.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were reported to be acclimated.

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Study Citation:	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).
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; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	11799635

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 midge per test chamber with eight replicates per treatment and control group.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	The test conditions were described adequately and were appropriate for organisms health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth measured by taking ash-free dry weights.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. On day 0, lengths were measured and representative ash-free dry weights were taken from a group of test organisms. Ash-free dry weights were also taken at the end of the 10-day study.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was described adequately and can be found in Appendix 21. Shapiro-Wilk’s test and Bartlett equality of variance tests were used.
	Metric 22: Reporting of Data	High	Growth was reported for all test concentrations and control in Table 10. Percent inhibition of growth was reported in Table 11. Individual replicate data is reported in Appendix 20.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Measures of variability were provided.

Additional Comments: This evaluation is for a 10d spiked sediment test using o-DCB and *C. dilutus* larvae. NOEC and LOEC values were reported.

Overall Quality Determination High

Study Citation:	Agroscience., E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).
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; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	11799635

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN in the certificate of analysis provided in Appendix 2.
Metric 2:	Test Substance Source	High	The test substance was analytically verified as reported in the certificate of analysis provided in Appendix 2.
Metric 3:	Test Substance Purity	High	The purity of the non-radiolabeled o-DCB was reported to be 99.7%. The radiolabeled test substance was reported to have a radiochemical purity of 97.0 area%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of both a negative control and a solvent control.
Metric 5:	Negative Control Response	High	The negative control and solvent control responses were reported in Appendix 9 for the range finding test.
Metric 6:	Randomized Allocation	Low	It was not reported how organisms were allocated into study groups in the range finding test.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	It was reported that the range finding test was conducted under intermittent-renewal systems. Little detail was provided on what this entailed. Preparation of the test sediment was not well described for the range-finder.
Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the exposure administration in the range-finding test.
Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured in the range-finding test. Only nominal concentrations were reported.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 10 days, which is in accordance with EPA test guidelines for spiked sediment tests.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure concentrations that were spaced quite far apart, but this was appropriate for a range-finding test.
Metric 12:	Testing at or Below Solubility Limit	N/A	This was a sediment test.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Test organisms were reported to originally be from Aquatic Bio Systems in Fort Collins, CO. A culture was maintained in-house at Eurofins-Easton aquatic ecotoxicology facility. Third-instar larvae were used at test initiation.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated for the range-finding test.

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Study Citation:	Agroscience., E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (Chironomus dilutus) using spiked whole sediment (o-DCB Test Order).
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; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	11799635

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 midges per test chamber with four replicates per treatment and control group in the range-finding test.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Test conditions for the range-finding test were reported to be similar to the definitive test, but little other information was provided.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest– mortality in terms of mean surviving organisms and mean percent survival.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. If an organism was immobile at the end of the test, it was considered dead.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little information was provided on acclimation. It was also only stated that the test conditions for the range-finding test were similar to the definitive test.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical methods for the range-finding test were not described in detail, but could be conducted from the data provided in Appendix 9.
	Metric 22: Reporting of Data	High	Survival was reported for all test concentrations and the control in Appendix 9.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Measures of variability were provided.
Additional Comments:	This evaluation is for a 10d spiked sediment test using o-DCB and <i>C. dilutus</i> larvae. This evaluation was for the range-finding test conducted prior to the definitive test. Survival was determined at the end of the 10-day test period.		

Overall Quality Determination

Medium

Study Citation:	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked whole sediment (o-DCB Test Order).		
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; <i>Chironomus dilutus</i> ; Larvae		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	11799635		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN in the certificate of analysis provided in Appendix 2.
Metric 2:	Test Substance Source	High	The test substance was analytically verified as reported in the certificate of analysis provided in Appendix 2.
Metric 3:	Test Substance Purity	High	The purity of the non-radiolabeled o-DCB was reported to be 99.7%. The radiolabeled test substance was reported to have a radiochemical purity of 97.0 area%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of both a negative control and a solvent control.
Metric 5:	Negative Control Response	High	The negative control and solvent control responses were reported in Table 10 and in Appendix 19 for survival. No significant difference was found between the negative control and the solvent control.
Metric 6:	Randomized Allocation	Medium	The midge larvae were reported to be randomly distributed into test groups and controls.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	A flow-through diluter system was used for the test system. Test compartments were 300mL beakers with about 100mL of test or control sediment and 175mL of overlying water. The preparation of the test sediment was reported in adequate detail.
Metric 8:	Consistency of Exposure Administration	High	The exposures were administered consistently across study groups. Test chambers were the the same across groups along with the amount of test sediment. Environmental conditions were the same across test groups, and the test substance concentrations were monitored for the duration of the study.
Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were analyzed at several time points for the duration of the test (days 0, 5, and 10). Overlying water, pore water, and sediment were all measured at these times. LSC was used to confirm test concentrations.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 10 days, which is in accordance with EPA test guidelines for spiked sediment tests.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure concentrations, which was adequate for a response.
Metric 12:	Testing at or Below Solubility Limit	N/A	This was a sediment test.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Test organisms were reported to originally be from Aquatic Bio Systems in Fort Collins, CO. A culture was maintained in-house at Eurofins-Easton aquatic ecotoxicology facility. Third-instar larvae were used at test initiation.
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Study Citation:	Agroscience,, E.E., LLC, (2024). o-Dichlorobenzene: A 10-day toxicity test with the midge (Chironomus dilutus) using spiked whole sediment (o-DCB Test Order).			
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; <i>Chironomus dilutus</i> ; Larvae			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	11799635			
Domain	Metric	Rating	Comments	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Test organisms were reported to be acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 midge per test chamber with eight replicates per treatment and control group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The test conditions were described adequately and were appropriate for organism health.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest– mortality in terms of mean surviving organisms. Ten-day LC50 values were determined.	
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups. If an organisms was immobile at the end of the test, it was considered dead.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical analysis was described adequately and can be found in Appendix 21. Survival was analyzed using Fisher’s Exact test.	
	Metric 22: Reporting of Data	High	Survival was reported for all test concentrations and control in Table 10. Percent inhibition of survival was reported in Table 11. Individual replicate data is reported in Appendix 19.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Measures of variability were shown.	
Additional Comments:	This evaluation is for a 10d spiked sediment test using o-DCB and C. dilutus larvae. A 10-day LC50 value of >25mg/kg was determined.			
Overall Quality Determination		High		

Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae		
Health Outcome:	Reproductive/Teratogenic		
Chemical:	o-Dichlorobenzene		
HERO ID:	12834309		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN in the text and in the certificate of analysis in Appendix 2.
	Metric 2: Test Substance Source	High	The non-radiolabeled o-DCB was from Sigma-Aldrich, and the radiolabeled o-DCB was from Eurofins-Selcia. Certificates of analysis were provided for each in Appendix 2.
	Metric 3: Test Substance Purity	High	The purity of the the non-radiolabeled test substance was reported to be 99.7%, and the radiochemical purity of the radiolabeled test substance was 97%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	The study reported the use of both a negative control and a solvent control.
	Metric 5: Negative Control Response	High	The negative control responses for reproduction are reported in Table 12 for both the negative and solvent controls.
	Metric 6: Randomized Allocation	Medium	It was reported first instar larvae were impartially placed into test chambers at test initiation.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The preparation of the test substance was reported in detail. The test system was an intermittent flow-through system that delivered two water volume replacements per day.
	Metric 8: Consistency of Exposure Administration	High	All test chambers were 300mL with 100mL of test sediment and 175mL of overlying water. Test conditions were consistent across all study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations in the sediment, pore water, and overlying water were monitored on days 0, 16, and 42 (50 for the solvent control). Stock solutions were also analyzed. Measurements were conducted via HPLC/UV as well as LSC. Test concentrations and stock solution concentrations as well as pore and overlying water concentrations are reported in Tables 1-8.
	Metric 10: Exposure Duration and Frequency	High	The test duration was reported to be 42 days (50 days for the solvent control only based on patterns and timing of observed emergence). This was adequate to observe any outcomes of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups with adequate spacing to observe any outcomes of interest.
	Metric 12: Testing at or Below Solubility Limit	N/A	This exposure was conducted via sediment.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The midges were from cultures maintained by Eurofins-Easton. First instar larvae (about 4 days of age) were used for the test.

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Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.			
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days			
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae			
Health Outcome:	Reproductive/Teratogenic			
Chemical:	o-Dichlorobenzene			
HERO ID:	12834309			
Domain	Metric	Rating	Comments	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Culturing conditions were described in detail and were similar to testing conditions, including the same dilution/culturing water and similar temperatures.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 16 organisms per test chamber with 12 original replicates per test concentration. An additional four replicates were added on day 6 to allow for male and female emergence near the same time, as males tend to emerge before females.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were reported and were conducive to the health of the test organisms.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest—reproduction outcomes in terms of total number of mated females per treatment group, number of primary egg masses per treatment group, number of primary egg masses per mated female, mean time to oviposition, average number of eggs per primary egg mass, average number of eggs per mated female, average percent hatch of primary egg masses per treatment group, and sex ratio.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. During the time of expected emergence (day 15 onward), the sex and number of fully emerged midges observed were recorded daily. The midges were then placed in reproduction/oviposition chambers, and each chamber was monitored daily for egg mass deposition. Primary egg masses were collected and the number of eggs hatched were monitored for six days.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were described clearly for each outcome assessed.	
	Metric 22: Reporting of Data	High	Reproductive outcomes were reported in Table 12 for all treatment groups and the controls.	
	Metric 23: Explanation of Unexpected Outcomes	High	The study did not report any unexpected outcomes, and variability was reported in the tables.	
Additional Comments:	This evaluation was for the reproduction outcomes reported here. Outcomes included total number of mated females per treatment group, total number of primary egg masses per treatment group, number of primary egg masses per mated female, mean time to oviposition, average number of eggs per primary egg mass, average number of eggs per mated female, average percent hatch of primary egg masses per treatment group, and sex ratio of emerging adults.			

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Study Citation: Agrosience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (*Chironomus dilutus*) using spiked sediment.
Duration: Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path: Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Invertebrate; Arthropods; *Chironomus dilutus*; Larvae
Health Outcome: Reproductive/Teratogenic
Chemical: o-Dichlorobenzene
HERO ID: 12834309

Domain	Metric	Rating	Comments
Overall Quality Determination		High	

Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	12834309		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The o-DCB was identified by CASRN in the text and in the certificate of analysis in Appendix 2.
	Metric 2: Test Substance Source	High	The non-radiolabeled o-DCB was from Sigma-Aldrich, and the radiolabeled o-DCB was from Eurofins-Selcia. Certificates of analysis were provided for each in Appendix 2.
	Metric 3: Test Substance Purity	High	The purity of the the non-radiolabeled test substance was reported to be 99.7%, and the radiochemical purity of the radiolabeled test substance was 97%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	The study reported the use of both a negative control and a solvent control.
	Metric 5: Negative Control Response	High	The negative control responses for mortality are reported in Tables 11 and 13 for both the negative and solvent controls.
	Metric 6: Randomized Allocation	Medium	It was reported first instar larvae were impartially placed into test chambers at test initiation.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The preparation of the test substance was reported in detail. The test system was an intermittent flow-through system that delivered two water volume replacements per day.
	Metric 8: Consistency of Exposure Administration	High	All test chambers were 300mL with 100mL of test sediment and 175mL of overlying water. Test conditions were consistent across all study groups.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations in the sediment, pore water, and overlying water were monitored on days 0, 16, and 42 (50 for the solvent control). Stock solutions were also analyzed. Measurements were conducted via HPLC/UV as well as LSC. Test concentrations and stock solution concentrations as well as pore and overlying water concentrations are reported in Tables 1-8.
	Metric 10: Exposure Duration and Frequency	High	The test duration was reported to be 42 days (50 days for the solvent control only based on patterns and timing of observed emergence). This was adequate to observe any outcomes of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups with adequate spacing to observe any outcomes of interest.
	Metric 12: Testing at or Below Solubility Limit	N/A	This exposure was conducted via sediment.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The midges were from cultures maintained by Eurofins-Easton. First instar larvae (about 4 days of age) were used for the test.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Culturing conditions were described in detail and were similar to testing conditions, including the same dilution/culturing water and similar temperatures.

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Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	12834309

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 16 organisms per test chamber with 12 original replicates per test concentration. An additional four replicates were added on day 6 to allow for male and female emergence near the same time, as males tend to emerge before females.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were reported and were conducive to the health of the test organisms.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest—mortality reported in terms of a 16d LC50 value and a mean time to death.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Organisms were assessed daily for mortality.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described clearly for each outcome assessed.
	Metric 22: Reporting of Data	High	Mortality data for the 16d sampling period was reported in Table 11 for all treatment levels and for the control groups. Table 13 contains the data for the mean time of death in days for all treatment groups and the controls.
	Metric 23: Explanation of Unexpected Outcomes	High	The study did not report any unexpected outcomes, and variability was reported in the tables.

Additional Comments: This evaluation was for the mortality outcome reported here. Authors reported a 16d LC50 value as well as a mean time to death at the end of the study.

Overall Quality Determination High

Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	12834309		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The o-DCB was identified by CASRN in the text and in the certificate of analysis in Appendix 2.
Metric 2:	Test Substance Source	High	The non-radiolabeled o-DCB was from Sigma-Aldrich, and the radiolabeled o-DCB was from Eurofins-Selcia. Certificates of analysis were provided for each in Appendix 2.
Metric 3:	Test Substance Purity	High	The purity of the the non-radiolabeled test substance was reported to be 99.7%, and the radiochemical purity of the radiolabeled test substance was 97%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The study reported the use of both a negative control and a solvent control.
Metric 5:	Negative Control Response	High	The negative control responses development/growth are reported in Tables 11 and 13 for both the negative and solvent controls.
Metric 6:	Randomized Allocation	Medium	It was reported first instar larvae were impartially placed into test chambers at test initiation.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The preparation of the test substance was reported in detail. The test system was an intermittent flow-through system that delivered two water volume replacements per day.
Metric 8:	Consistency of Exposure Administration	High	All test chambers were 300mL with 100mL of test sediment and 175mL of overlying water. Test conditions were consistent across all study groups.
Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations in the sediment, pore water, and overlying water were monitored on days 0, 16, and 42 (50 for the solvent control). Stock solutions were also analyzed. Measurements were conducted via HPLC/UV as well as LSC. Test concentrations and stock solution concentrations as well as pore and overlying water concentrations are reported in Tables 1-8.
Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 42 days (50 days for the solvent control only based on patterns and timing of observed emergence). This was adequate to observe any outcomes of interest.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups with adequate spacing to observe any outcomes of interest.
Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was conducted via sediment.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	The midges were from cultures maintained by Eurofins-Easton. First instar larvae (about 4 days of age) were used for the test.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Culturing conditions were described in detail and were similar to testing conditions, including the same dilution/culturing water and similar temperatures.
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Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (Chironomus dilutus) using spiked sediment.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	12834309

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 16 organisms per test chamber with 12 original replicates per test concentration. An additional four replicates were added on day 6 to allow for male and female emergence near the same time, as males tend to emerge before females.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were reported and were conducive to the health of the test organisms.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest—development and growth in terms of number of emerged adults, mean development time, and mean development rate at day 42/50. Ash free dry weights were determined at day 16.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Organisms were assessed at day 16 for the ash free dry weight. Organisms were assessed for emergence daily starting on day 15.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described clearly for each outcome assessed.
	Metric 22: Reporting of Data	High	Development/growth outcomes were reported in Tables 11 and 13. Data was reported for all test concentrations and for the negative and solvent controls.
	Metric 23: Explanation of Unexpected Outcomes	High	The study did not report any unexpected outcomes, and variability was reported in the tables.
Additional Comments:	This evaluation was for the development/growth outcomes reported here. Outcomes include number of emerged midges, mean emergence rate, mean developmental time, and mean development rate for the entire 42/50 day study. At 16 days, larvae were sampled and ash free dry weights were determined.		

Overall Quality Determination

High

Study Citation:	Agroscience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (<i>Chironomus dilutus</i>) using spiked sediment.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Chironomus dilutus</i> ; Larvae		
Health Outcome:	Behavioral		
Chemical:	o-Dichlorobenzene		
HERO ID:	12834309		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The o-DCB was identified by CASRN in the text and in the certificate of analysis in Appendix 2.
Metric 2:	Test Substance Source	High	The non-radiolabeled o-DCB was from Sigma-Aldrich, and the radiolabeled o-DCB was from Eurofins-Selcia. Certificates of analysis were provided for each in Appendix 2.
Metric 3:	Test Substance Purity	High	The purity of the the non-radiolabeled test substance was reported to be 99.7%, and the radiochemical purity of the radiolabeled test substance was 97%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	The study reported the use of both a negative control and a solvent control.
Metric 5:	Negative Control Response	Medium	Authors mentioned there were no unusual observations of organisms avoiding the sediment during the test. However, specific mention of control behavior was not reported.
Metric 6:	Randomized Allocation	Medium	It was reported first instar larvae were impartially placed into test chambers at test initiation.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The preparation of the test substance was reported in detail. The test system was an intermittent flow-through system that delivered two water volume replacements per day.
Metric 8:	Consistency of Exposure Administration	High	All test chambers were 300mL with 100mL of test sediment and 175mL of overlying water. Test conditions were consistent across all study groups.
Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations in the sediment, pore water, and overlying water were monitored on days 0, 16, and 42 (50 for the solvent control). Stock solutions were also analyzed. Measurements were conducted via HPLC/UV as well as LSC. Test concentrations and stock solution concentrations as well as pore and overlying water concentrations are reported in Tables 1-8.
Metric 10:	Exposure Duration and Frequency	High	The test duration was reported to be 42 days (50 days for the solvent control only based on patterns and timing of observed emergence). This was adequate to observe any outcomes of interest.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups with adequate spacing to observe any outcomes of interest.
Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was conducted via sediment.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	The midges were from cultures maintained by Eurofins-Easton. First instar larvae (about 4 days of age) were used for the test.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Culturing conditions were described in detail and were similar to testing conditions, including the same dilution/culturing water and similar temperatures.
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Study Citation: Agrosience,, E.E., LLC, (2025). o-Dichlorobenzene: A life cycle toxicity test with the midge (*Chironomus dilutus*) using spiked sediment.
Duration: Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path: Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Invertebrate; Arthropods; *Chironomus dilutus*; Larvae
Health Outcome: Behavioral
Chemical: o-Dichlorobenzene
HERO ID: 12834309

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 16 organisms per test chamber with 12 original replicates per test concentration. An additional four replicates were added on day 6 to allow for male and female emergence near the same time, as males tend to emerge before females.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Organism housing and environmental conditions were reported and were conducive to the health of the test organisms.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest—abnormal behavior (leaving the sediment).
	Metric 18: Consistency of Outcome Assessment	High	Observations were made daily throughout the test.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	There were no differences between test groups in abnormal behavior (none observed), so statistical analysis was not necessary.
	Metric 22: Reporting of Data	Medium	Authors just mentioned in the text that there were no unusual observations of organisms avoiding the sediment during the test. There was no raw data included for this outcome assessment.
	Metric 23: Explanation of Unexpected Outcomes	High	The study did not report any unexpected outcomes.

Additional Comments: This evaluation is for the behavioral assessment.

Overall Quality Determination

High

Study Citation:	Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. <i>Aquatic Toxicology</i> 8(3):163-174.			
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; <i>Daphnia magna</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	1486051			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	High	Test substance purity was reported as at least 97%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control group was reported.	
	Metric 5: Negative Control Response	High	Control group mortality was less than 10%, as reported in the text.	
	Metric 6: Randomized Allocation	Low	The paper does not report on how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation methods were briefly given in this paper and another reference was cited, which was reviewed. Appropriate measures were taken to minimize the loss of test substances.	
	Metric 8: Consistency of Exposure Administration	High	The study cites another reference, which was reviewed. Exposure administration was consistent among groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and nominal concentrations of the treatment groups were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The acute daphnid study had a 48-hr duration.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was reported that at least 5 exposure groups were tested, but no information was provided on the exposure concentrations and the spacing of exposure levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the solubility limit. It was reported that a saturated aqueous stock solution was prepared and then diluted to provide 5 exposure concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Daphnia of similar size (approximately 1.5 m in length, 4-6 days old) were chosen as per the cited reference. Daphnia were reported to be cultured in the performing laboratory.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Per the cited reference, daphnids were held in distilled water with a pH of 6.5, but it does not report whether pretreatment conditions were the same for control and exposed groups.	

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Study Citation:	Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. <i>Aquatic Toxicology</i> 8(3):163-174.
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; <i>Daphnia magna</i> ; Juvenile
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	1486051

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	The cited reference reported the use of 10 animals per vial and 2-4 replicates per test concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not reported other than temperature. Loading biomass exceeds (10 animals per 33 ml vial) the recommendation in the EPA Series 850 Ecological Effects Test Guidelines.
	Metric 17: Outcome Assessment Methodology	High	The mortality assessment was conducted at 24 h and 48 h using Stereostar zoom microscope.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was done at 24 h and 48 h and appears to be consistent across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information on environmental conditions across treatment groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information on health outcomes unrelated to exposure was provided.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was conducted. LC50 values, slope factors, and 95% confidence limits were calculated by the graphical method of Litchfield and Wilcoxon (1948).
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. Only LC 50 values were presented.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.
Additional Comments:	The study did not report exposure concentrations (nominal or measured) and did not provide dose-response data. This evaluation is for the acute toxicity of o-DCB to <i>Daphnia</i> .		

Overall Quality Determination

Uninformative

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.
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; <i>Daphnia magna</i> ; Juvenile
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.
	Metric 3: Test Substance Purity	Low	The percent purity was not reported, but it was reported that all chemicals were analytical grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	Low	Limited details were given in the paper with respect to a control. Another cited methodology was needed to assess this metric and was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 5: Negative Control Response	Low	The biological response of the negative control group for the test substance was not reported.
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study reported that tests were carried out in closed systems, but major details regarding test media preparation were not given in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration details are not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured at the beginning and the end of the short-term tests, but they were not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 10: Exposure Duration and Frequency	High	This was an acute immobilization test conducted for 24 h. According to OECD guidelines for Test No. 202, determination of EC 50 at 24 h is optional.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups and spacing of exposure levels in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
	Metric 12: Testing at or Below Solubility Limit	Low	No details were provided to determine whether exposure concentrations exceeded the water solubility limit.

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Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.
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; <i>Daphnia magna</i> ; Juvenile
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported, but the test organism was appropriate for evaluating the outcome. Another cited methodology needed to assess this metric was unavailable, therefore this metric score reflects the amount of details provided in the study being reviewed.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates were not reported. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not reported in the paper. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 18:	Consistency of Outcome Assessment	Low	No details were provided regarding the execution of the study protocol for outcome assessment.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Another cited methodology needed to assess this metric was unavailable, therefore, this metric score reflects the amount of details provided in the study being reviewed.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information presented to suggest differences among treatment groups were due to exposure unrelated reasons.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described well.
Metric 22:	Reporting of Data	Low	Immobilization data for treatments groups and controls were not reported. Only 24h IC 50 values were reported.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

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Study Citation: Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12(2):253-262.
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: Immobilization
Chemical: o-Dichlorobenzene
HERO ID: 4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Additional Comments:	The source of test organisms, exposure concentrations (nominal or measured), spacing of exposure levels, and control response were not reported. Only 24h IC 50 values along with confidence intervals were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Deneer, J. W., Sinnige, T. L., Seinen, W., Hermens, J. L. (1988). The joint acute toxicity to <i>Daphnia magna</i> of industrial organic chemicals at low concentrations. <i>Aquatic Toxicology</i> 12(1):33-38.			
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; <i>Daphnia magna</i> ; Juvenile			
Health Outcome:	Immobilization			
Chemical:	o-Dichlorobenzene			
HERO ID:	660799			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	Chemical source and analytical verification were not reported.	
	Metric 3: Test Substance Purity	Low	Chemical purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	The use of a control was not reported although it is implied for the calculation of an IC50.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	No random allocation was reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was very little information provided regarding experimental set up.	
	Metric 8: Consistency of Exposure Administration	Medium	No inconsistencies were reported, but again not many details on the experimental system were provided.	
	Metric 9: Measurement of Test Substance Concentration	Low	The test chemical was not measured.	
	Metric 10: Exposure Duration and Frequency	High	A standard acute duration was used.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The concentration range was sufficient to assess the outcome, but values weren't reported.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The IC50 value was well below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	Not much information was given about the test organisms, other than they were less than a day old at the start of the experiment.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Acclimatization conditions were not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There was no mention of the number of test organisms used, only a methods citation was provided.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Test conditions were not reported.	

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Study Citation:	Deneer, J. W., Sinnige, T. L., Seinen, W., Hermens, J. L. (1988). The joint acute toxicity to <i>Daphnia magna</i> of industrial organic chemicals at low concentrations. <i>Aquatic Toxicology</i> 12(1):33-38.
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; <i>Daphnia magna</i> ; Juvenile
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	660799

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Low	Outcome assessment methodology was not reported.
	Metric 18: Consistency of Outcome Assessment	Medium	Outcomes for seven tests were reported with an almost 3x factor between lowest and highest values.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	No confounding variables were reported other than various batches of daphnids were used.
	Metric 20: Outcomes Unrelated to Exposure	Medium	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical methods were not discussed.
	Metric 22: Reporting of Data	Medium	Intended data reporting was adequate. No raw data were shown.
	Metric 23: Explanation of Unexpected Outcomes	Medium	There was some variability in the results that was mentioned but not thoroughly discussed.

Additional Comments: p-DCB IC50 was provided from another source. It was not used in this study exposure.

Overall Quality Determination

Low

Study Citation:	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.
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; <i>Daphnia magna</i> ; IRCHA; Juvenile
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	773229

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The chemical source and analytical verification were not reported.
	Metric 3: Test Substance Purity	Low	The chemical purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	The use of negative controls was reported.
	Metric 5: Negative Control Response	Low	The control response was not reported.
	Metric 6: Randomized Allocation	Low	The use of random allocation was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Media preparation was reported in some detail, but details specific to this test chemical were vague.
	Metric 8: Consistency of Exposure Administration	Medium	No inconsistencies were reported, but details were sparse. Some test chambers were covered and some were not. Covered chambers were only fed on water renewal days at higher rates, whereas uncovered chambers were fed daily.
	Metric 9: Measurement of Test Substance Concentration	Medium	Some measurements were reported for the test chemical.
	Metric 10: Exposure Duration and Frequency	Medium	The duration was shorter than usual, but it was sufficient to obtain a study range for the chronic test.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of the groups were not reported as the actual test concentrations were not reported. However, a concentration range was reported.
	Metric 12: Testing at or Below Solubility Limit	Medium	Extra steps were required to dissolve the chemical. Limited details of the chemical concentrations gave uncertainty to the actual concentration levels.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	Organisms were reported to be from in house cultures and were 24h old at the start of the study.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were reasonably documented.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect.

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Study Citation:	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.
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; <i>Daphnia magna</i> ; IRCHA; Juvenile
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	773229

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test organism housing, environmental conditions, food, water, and nutrients were appropriate.
	Metric 17: Outcome Assessment Methodology	Medium	The method of observing the acute test was not reported. EC values were reported indicating immobilization was used. Authors also reported removing dead organisms and those that were not moving.
	Metric 18: Consistency of Outcome Assessment	Medium	Limited details were provided, so it was difficult to assess consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	No raw data was reported for the acute test. Methods for obtaining ECx values were not reported. Only statistical methods for the NOEC values were reported.
	Metric 22: Reporting of Data	Low	No raw data was reported for acute test. Only ECx values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation is for the acute ECx values for o-dichlorobenzene.

Overall Quality Determination

Medium

Study Citation:	Pawlisz, A. V., Peters, R. H. (1995). Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . Environmental Science & Technology 29(3):613-621.
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; <i>Daphnia magna</i> ; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	2777011; Linked HERO ID(s): 2777011, 5255675

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The source was reported, but chemical verification was not.
	Metric 3: Test Substance Purity	High	Chemical purity was reported as >98%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	The use of a negative control was reported.
	Metric 5: Negative Control Response	Low	No control response was reported.
	Metric 6: Randomized Allocation	Low	The use of randomized allocation of organisms to study groups was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The test system was adequately described, and preparation of the test media was described in detail.
	Metric 8: Consistency of Exposure Administration	High	No inconsistencies were reported.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured in water before and after the exposure.
	Metric 10: Exposure Duration and Frequency	High	The duration was appropriate for the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four treatment concentrations plus a control. The exposure concentrations used were suitable for assessment of the desired outcome.
	Metric 12: Testing at or Below Solubility Limit	High	The high concentration was well below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	Age of the daphnids used was not reported and very few other details were provided about the test organisms.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	No pretreatment was reported but details describing daphnid culture conditions were provided.
	Metric 15: Number of Organisms and Replicates per Group	Low	No replicates were reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Limited details were provided on conditions during the test. The number of daphnids per test vial seemed appropriate to determine an accurate result.

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Study Citation: Pawlisz, A. V., Peters, R. H. (1995). Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environmental Science & Technology* 29(3):613-621.
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*; Adult
Health Outcome: ADME (biotransformation)
Chemical: o-Dichlorobenzene
HERO ID: 2777011; Linked HERO ID(s): 2777011, 5255675

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	It wasn't clear if they analyzed all exposed daphnids or just the ones that were immobilized.
	Metric 18: Consistency of Outcome Assessment	Medium	Downgraded based on uncertainty of metric 17.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences among test organisms that could impact the exposure outcome.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The statistical methods seemed appropriate and were described adequately.
	Metric 22: Reporting of Data	Medium	Actual mortalities were not reported. Sublethal body burden results were shown in Figures 1-4.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported for o-DCB. Measures of variability were included in the figures.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2795-2800.			
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	2779031			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The o-dichlorobenzene was identified by name only.	
	Metric 2: Test Substance Source	Low	The radiolabeled o-dichlorobenzene was reported to be from Sigma. The unlabeled forms of the chemicals were reported to be from either Sigma or Beckman Chemicals, but it was not specified which company the unlabeled o-dichlorobenzene came from. It was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity of the radiolabeled form was reported to be >98%, but the unlabeled purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported that two vials without any chemicals were used as a negative control.	
	Metric 5: Negative Control Response	Low	The response of the negative control was not reported.	
	Metric 6: Randomized Allocation	Low	It was not reported how the <i>D. magna</i> were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The exposure system was reported to be a static system with 20mL screw top vials with 18mL of test solution. Solutions were not renewed for the duration of the study. Radiolabeled chemical was diluted with the nonradioactive carrier to increase the amount of available. The test solutions were prepared by placing an aliquot of the chemical into a volumetric flask containing reconstituted water, the flask was then sealed and wrapped in aluminum foil and shaken gently by hand. This was then placed on a magnetic heater at 23C for 24-72h in order for the chemical to saturate or dissolve. Exact measurements of the test substance used were not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	All test chambers were 20mL vials with 18mL of test solution. Fifteen parthenogenic females were used. Organisms were all kept at 20C. The test concentrations were not reported, though other literature was cited for determining the test concentrations. The age of the organisms was not reported, but it was reported they were different sizes, creating consistency issues.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Study authors reported the test concentrations were measured after the preparation and after each test run with a Wallac LKB scintillation counter. Radioactivities were then converted into chemicals concentrations and expressed as millimole per liter. Loss of contaminant was reported to be about 10%. Actual measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 48h, which is typical of an acute <i>Daphnia</i> study.	

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Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . Environmental Science & Technology 27(13):2795-2800.		
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	2779031		
Domain	Metric	Rating	Comments
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors did not report the number of exposure groups or the spacing of the exposure groups. But they did cite published sources for the 48h LC50 in which the concentrations were based off. Depending what is provided in this source, this metric may need to be rated unacceptable. Veith, G. D.; Austin, N. M.; Morris, R.T. Water Res. 1979, 13, 43-47.
	Metric 12: Testing at or Below Solubility Limit	Low	Because exposure concentrations were not reported, it cannot be determined if any were above the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from cultures from the performing laboratory that originated from the Environmental Biology Annex I at the University of Guelph in Ontario, Canada. However, the age of the organisms was not reported, and it was reported different sizes were used, so this could indicate different ages were used.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	It was reported the <i>Daphnia</i> were acclimated to test conditions prior to the start of the study, but exact period of acclimation was not reported.
	Metric 15: Number of Organisms and Replicates per Group	Medium	It was reported 15 organisms were allocated into eight test vials, and that each test concentration was replicated 4-7 times.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	<i>Daphnia</i> were kept at 20C with a 16L:8D photoperiod. In culture, there were 75 organisms per 1000mL of culture water. The culture water was reported to be reconstituted water, but properties of the water were not reported. The <i>Daphnia</i> were also reported to be fed "green water" daily while in culture. The "green water" was reported to be algal suspension from goldfish tanks, and the it was reported cultures received enough to keep them slightly green. An exact concentration was not reported. The source of the algae in the lack of information regarding the concentration is concerning.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment was for determining the body burden in dead, immobilized, and unaffected <i>D. magna</i> . In the process of this, the number of dead <i>D. magna</i> were tallied, though mortality itself was not the outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	<i>D. magna</i> were observed under a dissecting scope. If the organism did not show signs of movement in the appendages, digestive tract, and heart, it was considered to be dead. The number of observations of dead organisms is reported in Fig. 1.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There were concerns about the environmental conditions regarding the food source and the concentration of the food the <i>Daphnia</i> were being fed.

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Study Citation: Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environmental Science & Technology* 27(13):2795-2800.

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: Mortality

Chemical: o-Dichlorobenzene

HERO ID: 2779031

Domain	Metric	Rating	Comments
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Statistical analysis was not conducted for mortality. The number of dead organisms observed was reported in Fig 1.
	Metric 22: Reporting of Data	Low	The number of dead organisms observed was reported in Fig 1, but this was not necessarily the outcome of interest. Control results were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported for the mortality outcome.

Additional Comments: This portion of the study was on the effect of o-chlorobenzene on *D. magna* mortality. The number of mortalities observed was reported in Figure 1, though this was not necessarily an outcome of interest. This portion received an unacceptable rating due to lack of statistics. The study also did not provide the exposure concentrations, but instead cited another source for the LC50 value the exposure concentrations were based on. This could potentially change Metric 11 from low to unacceptable depending on what is reported in the cited source listed below. Veith, G. D.; Austin, N. M.; Morris, R.T. *Water Res.* 1979, 13, 43-47.

Overall Quality Determination

Uninformative

Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2795-2800.
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	2779031

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The o-dichlorobenzene was identified by name only.
	Metric 2: Test Substance Source	Low	The radiolabeled o-dichlorobenzene was reported to be from Sigma. The unlabeled forms of the chemicals were reported to be from either Sigma or Beckman Chemicals, but it was not specified which company the unlabeled o-dichlorobenzene came from. It was not reported to be analytically verified.
	Metric 3: Test Substance Purity	High	The purity of the radiolabeled form was reported to be >98%, but the unlabeled purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported that two vials without any chemicals were used as a negative control.
	Metric 5: Negative Control Response	Low	The response of the negative control was not reported.
	Metric 6: Randomized Allocation	Low	It was not reported how the <i>D. magna</i> were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The exposure system was reported to be a static system with 20mL screw top vials with 18mL of test solution. Solutions were not renewed for the duration of the study. Radio-labeled chemical was diluted with the nonradioactive carrier to increase the amount of available. The test solutions were prepared by placing an aliquot of the chemical into a volumetric flask containing reconstituted water, the flask was then sealed and wrapped in aluminum foil and shaken gently by hand. This was then placed on a magnetic heater at 23C for 24-72h in order for the chemical to saturate or dissolve. Exact measurements of the test substance used were not reported.
	Metric 8: Consistency of Exposure Administration	Medium	All test chambers were 20mL vials with 18mL of test solution. Fifteen parthenogenic females were used. Organisms were all kept at 20C. The test concentrations were not reported, though other literature was cited for determining the test concentrations. The age of the organisms was not reported, but it was reported they were different sizes, creating consistency issues.
	Metric 9: Measurement of Test Substance Concentration	Medium	Study authors reported the test concentrations were measured after the preparation and after each test run with a Wallac LKB scintillation counter. Radioactivities were then converted into chemicals concentrations and expressed as millimole per liter. Loss of contaminant was reported to be about 10%. Actual measurements were not reported.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 48h, which is typical of an acute <i>Daphnia</i> study.

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Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . Environmental Science & Technology 27(13):2795-2800.
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Immobilization
Chemical:	o-Dichlorobenzene
HERO ID:	2779031

Domain	Metric	Rating	Comments
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors did not report the number of exposure groups or the spacing of the exposure groups. But they did cite published sources for the 48h LC50 in which the concentrations were based off. Depending what is provided in this source, this metric may need to be rated unacceptable. Veith, G. D.; Austin, N. M.; Morris, R.T. Water Res. 1979, 13, 43-47.
	Metric 12: Testing at or Below Solubility Limit	Low	Because exposure concentrations were not reported, it cannot be determined if any were above the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from cultures from the performing laboratory that originated from the Environmental Biology Annex I at the University of Guelph in Ontario, Canada. However, the age of the organisms was not reported, and it was reported different sizes were used, so this could indicate different ages were used.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	It was reported the <i>Daphnia</i> were acclimated to test conditions prior to the start of the study, but exact period of acclimation was not reported.
	Metric 15: Number of Organisms and Replicates per Group	Medium	It was reported 15 organisms were allocated into eight test vials, and that each test concentration was replicated 4-7 times.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	<i>Daphnia</i> were kept at 20C with a 16L:8D photoperiod. In culture, there were 75 organisms per 1000mL of culture water. The culture water was reported to be reconstituted water, but properties of the water were not reported. The <i>Daphnia</i> were also reported to be fed "green water" daily while in culture. The "green water" was reported to be algal suspension from goldfish tanks, and the it was reported cultures received enough to keep them slightly green. An exact concentration was not reported. The source of the algae in the lack of information regarding the concentration is concerning.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment was for determining the body burden in dead, immobilized, and unaffected <i>D. magna</i> . In the process of this, the number of immobilized <i>D. magna</i> were tallied, though immobilization itself was not the outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	<i>D. magna</i> were observed under a dissecting scope. If the organism did not show signs of normal swimming movement, but did show signs of digestive and heart movement with some appendage movement it was considered immobilized.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There were concerns about the environmental conditions regarding the food source and the concentration of the food the <i>Daphnia</i> were being fed.

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Study Citation: Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environmental Science & Technology* 27(13):2795-2800.
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: Immobilization
Chemical: o-Dichlorobenzene
HERO ID: 2779031

Domain	Metric	Rating	Comments
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Statistical analysis was not conducted for immobilization. The number of immobilized organisms observed was reported in Fig 1.
	Metric 22: Reporting of Data	Low	The number of immobilized organisms observed was reported in Fig 1, but this was not necessarily the outcome of interest. Control results were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported for the immobilization outcome.

Additional Comments: This portion of the study was on the effect of o-chlorobenzene on *D. magna* immobilization. The number of immobilized organisms was observed and was reported in Figure 1, though this was not necessarily an outcome of interest. This portion received an unacceptable rating due to lack of statistics. The study also did not provide the exposure concentrations, but instead cited another source for the LC50 value the exposure concentrations were based on. This could potentially change Metric 11 from low to unacceptable depending on what is reported in the cited source listed below. Veith, G. D.; Austin, N. M.; Morris, R.T. *Water Res.* 1979, 13, 43-47.

Overall Quality Determination

Uninformative

Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2795-2800.
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	2779031

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The o-dichlorobenzene was identified by name only.
	Metric 2: Test Substance Source	Low	The radiolabeled o-dichlorobenzene was reported to be from Sigma. The unlabeled forms of the chemicals were reported to be from either Sigma or Beckman Chemicals, but it was not specified which company the unlabeled o-dichlorobenzene came from. It was not reported to be analytically verified.
	Metric 3: Test Substance Purity	High	The purity of the radiolabeled form was reported to be >98%, but the unlabeled purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported that two vials without any chemicals were used as a negative control.
	Metric 5: Negative Control Response	Low	The response of the negative control was not reported.
	Metric 6: Randomized Allocation	Low	It was not reported how the <i>D. magna</i> were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The exposure system was reported to be a static system with 20mL screw top vials with 18mL of test solution. Solutions were not renewed for the duration of the study. Radio-labeled chemical was diluted with the nonradioactive carrier to increase the amount of available. The test solutions were prepared by placing an aliquot of the chemical into a volumetric flask containing reconstituted water, the flask was then sealed and wrapped in aluminum foil and shaken gently by hand. This was then placed on a magnetic heater at 23C for 24-72h in order for the chemical to saturate or dissolve. Exact measurements of the test substance used were not reported.
	Metric 8: Consistency of Exposure Administration	Medium	All test chambers were 20mL vials with 18mL of test solution. Fifteen parthenogenic females were used. Organisms were all kept at 20C. The test concentrations were not reported, though other literature was cited for determining the test concentrations.
	Metric 9: Measurement of Test Substance Concentration	Medium	Study authors reported the test concentrations were measured after the preparation and after each test run with a Wallac LKB scintillation counter. Radioactivities were then converted into chemical concentrations and expressed as millimole per liter. Loss of contaminant was reported to be about 10%. Actual measurements were not reported.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 48h, which is typical of an acute <i>Daphnia</i> study.

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Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . Environmental Science & Technology 27(13):2795-2800.			
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	2779031			
Domain	Metric	Rating	Comments	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors did not report the number of exposure groups or the spacing of the exposure groups. But they did cite published sources for the 48h LC50 in which the concentrations were based off. Depending what is provided in this source, this metric may need to be rated unacceptable. Veith, G. D.; Austin, N. M.; Morris, R.T. Water Res. 1979, 13, 43-47.	
	Metric 12: Testing at or Below Solubility Limit	Low	Because exposure concentrations were not reported, it cannot be determined if any were above the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Medium	The test organisms were reported to be from cultures from the performing laboratory that originated from the Environmental Biology Annex I at the University of Guelph in Ontario, Canada. However, the age of the organisms was not reported, and it was reported different sizes were used, so this could indicate different ages were used.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	It was reported the <i>Daphnia</i> were acclimated to test conditions prior to the start of the study, but the exact period of acclimation was not reported.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	It was reported 15 organisms were allocated into eight test vials, and that each test concentrations was replicated 4-7 times.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	<i>Daphnia</i> were kept at 20C with a 16L:8D photoperiod. In culture, there were 75 organisms per 1000mL of culture water. The culture water was reported to be reconstituted water, but properties of the water were not reported. The <i>Daphnia</i> were also reported to be fed "green water" daily while in culture. The "green water" was reported to be algal suspension from goldfish tanks, and the it was reported cultures received enough to keep them slightly green. An exact concentration was not reported. The source of the algae in the lack of information regarding the concentration is concerning.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—accumulation of o-dichlorobenzene in <i>D. magna</i> tissue after 48h of exposure.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. All organisms were assessed at the end of the study and were determined to be unaffected, immobilized, or dead. They were examined under a dissecting scope for normal swimming movement, only movement of the digestive tract or heart, or no movement of those. They were then assessed for radioactivity.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There were concerns about the environmental conditions regarding the food source and the concentration of the food the <i>Daphnia</i> were being fed.	
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Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in <i>Daphnia magna</i> . <i>Environmental Science & Technology</i> 27(13):2795-2800.
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	2779031

Domain	Metric	Rating	Comments
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were described adequately in the "statistical analysis" section of the paper.
	Metric 22: Reporting of Data	Medium	Cumulative percent distributions of the body burdens of o-dichlorobenzene in dead, immobilized, and unaffected <i>D. magna</i> after the exposure are reported in Fig 1. Control results were not presented.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors explained unexpected outcomes sufficiently, and variability was reported in Table IV for SD of mean body burdens.

Additional Comments: This portion of the evaluation was on the body burden of o-dichlorobenzene in *D. magna* after 48h of exposure. Exposure concentrations were not reported, but a published source was cited for the 48h LC50 value the exposure concentrations were based on. This is why Metric 11 received a low ranking instead of an unacceptable, but this may need to change depending on the data provided in the sources listed below. ADME was selected as the outcome of interest. Veith, G. D.; Austin, N. M.; Morris, R.T. *Water Res.* 1979, 13, 43-47.

Overall Quality Determination

Medium

Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A test of the equipotency of internal burdens of nine narcotic chemicals using <i>Daphnia magna</i> . Environmental Science & Technology 27(13):2801-2806.		
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	5255675; Linked HERO ID(s): 2777011, 5255675		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test chemical was identified by name only.
Metric 2:	Test Substance Source	Low	It was reported that the chemicals were obtained from either Sigma or CHEMSYN chemical companies. It was not reported if the test chemical was analytically verified.
Metric 3:	Test Substance Purity	High	The purity was greater than 98%.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	A control was used.
Metric 5:	Negative Control Response	Low	The control response was not reported.
Metric 6:	Randomized Allocation	Low	It was not reported if the <i>D. magna</i> were randomly allocated to test vials.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	The test solutions were prepared in water. No carrier solvent was needed. During the exposure the vials were closed with screw caps. Authors reportedly tested different durations, concentrations and sized <i>D. magna</i> but details were lacking on how those specific parameters were incorporated into the experimental runs.
Metric 8:	Consistency of Exposure Administration	Low	Since there are some details lacking on the different parameters tested, there could have been some issues with consistency throughout the exposure.
Metric 9:	Measurement of Test Substance Concentration	Low	After preparation of the test solution (before the experiment start) the final concentration in the flask was checked by placing 50uL of test solution in a scintillation vial with 2mL of Ready Protein scintillation fluid. The measured radioactivity was divided by specific activity to give chemical concentration in millimole per liter. It was not mentioned what equipment was used to make the measurements. There was no mention of measuring concentrations during or at the end of the experimental runs. And again, authors only mentioned testing a range of concentrations based on the chemical's 48h LC50 value. No specific nominal concentrations were reported.
Metric 10:	Exposure Duration and Frequency	Low	Study authors did not specifically report the study duration, but it is inferred that the max exposure was 48 hours (Figure 1 text). It was also mentioned that some experiments had a duration of seconds (unsure of how many though).
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	Authors mentioned exposing <i>D. magna</i> to test concentrations ranging from 1/10th to the full 48-h LC50 level. However, the number of test concentrations was not reported, nor was the spacing of the test concentrations.
Metric 12:	Testing at or Below Solubility Limit	High	Study authors reported that the aqueous test solutions did not exceed the solubilities of the chemicals.

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Study Citation:	Pawlisz, A. V., Peters, R. H. (1993). A test of the equipotency of internal burdens of nine narcotic chemicals using <i>Daphnia magna</i> . Environmental Science & Technology 27(13):2801-2806.
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5255675; Linked HERO ID(s): 2777011, 5255675

Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	It was not reported where the <i>D. magna</i> came from. It was just mentioned that parthenogenic females were used. The <i>D. magna</i> used ranged in length from 0.4-3.2mm. The actual age of the organisms was also not reported. It is unclear if age differences were the reason for size differences.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the <i>D. magna</i> were acclimated to test conditions prior to the start. Before the test they were kept under constant environmental conditions, but it is not clear if those were the same conditions during the test.
	Metric 15: Number of Organisms and Replicates per Group	Low	Fifteen <i>Daphnia</i> were placed in each test vial. There was no mention of replicates per test concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Test conditions were not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessed was chemical accumulation. Methods were described adequately.
	Metric 18: Consistency of Outcome Assessment	High	There was no indication that the outcome was not assessed consistently.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	There was no mention of acclimating <i>D. magna</i> prior to the test start and conditions during the test were not reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information suggesting differences among <i>D. magna</i> between test groups in attrition or health issues that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	SAS statistical software was used for analyses. Sufficient details were given on the statistical analyses on page 2 of the pdf.
	Metric 22: Reporting of Data	Low	No raw data were reported. Table 1 (effects of parameters) and Figure 1 (48-h LC50 level) showed calculated results based on the data obtained. Figures 2 and 3 showed predicted body burdens. No results were shown for the controls.
	Metric 23: Explanation of Unexpected Outcomes	High	Measures of variability were shown in the figure and table.
Additional Comments:	This evaluation is for the assessment of chemical accumulation in <i>D. magna</i> . This study assessed nine different chemicals, including 1,2-dichlorobenzene. The authors mentioned experimenting with a range of concentrations, organism sizes, and test durations (seconds to days but not exceeding 48 hours). The details of these parameters were lacking. This study received an unacceptable rating due to the lack of reporting regarding the test concentrations used as well as the lack of information of the spacing of the test concentrations.		

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Study Citation: Pawlisz, A. V., Peters, R. H. (1993). A test of the equipotency of internal burdens of nine narcotic chemicals using *Daphnia magna*. Environmental Science & Technology 27(13):2801-2806.

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: ADME (biotransformation)

Chemical: o-Dichlorobenzene

HERO ID: 5255675; Linked HERO ID(s): 2777011, 5255675

Domain	Metric	Rating	Comments
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Overall Quality Determination **Uninformative**

Study Citation:	Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran <i>Ceriodaphnia cf. dubia</i> . Archives of Environmental Contamination and Toxicology 34(3):248-252.		
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; <i>Daphnia magna</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Immobilization		
Chemical:	o-Dichlorobenzene		
HERO ID:	651551		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by name and CAS #.
Metric 2:	Test Substance Source	Low	The chemical source and any analytical verification were not reported.
Metric 3:	Test Substance Purity	High	All chemicals were >=97 % pure.
Domain 2: Test Design			
Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control.
Metric 5:	Negative Control Response	Low	Use of controls was not reported nor was the control response.
Metric 6:	Randomized Allocation	Low	There was no mention of using random allocation.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	Little details were provided on the test system, though headspace was reduced to limit volatilization of the test substance. Preparation of the test concentrations was not described in great detail, but test concentrations were measured and reported to be within 20% of nominal. The concentrations measured were only at test initiation.
Metric 8:	Consistency of Exposure Administration	Low	No inconsistencies were reported, but there was not much information reported either.
Metric 9:	Measurement of Test Substance Concentration	Medium	Concentrations were only measured at the beginning of the test.
Metric 10:	Exposure Duration and Frequency	High	Exposure durations followed standard protocol.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of test concentrations and their spacing were not reported. Though US EPA methods were cited, it is possible deviations occurred that were not reported in this paper.
Metric 12:	Testing at or Below Solubility Limit	Medium	EC50 values were well below solubility, acetone was also added to aid in dissolving the chemical.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	Little information was provided the test organisms. The source of the <i>D. magna</i> was not reported. Warne 1996 was cited for maintenance conditions. Little was reported on test conditions. The age of the organisms was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	No acclimatization conditions were reported.
Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms and replicates per group were not reported.

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Study Citation: Rose, R. M., Warne, M. S., Lim, R. P. (1998). Quantitative structure-activity relationships and volume fraction analysis for nonpolar necrotic chemicals to the Australian cladoceran *Ceriodaphnia cf. dubia*. Archives of Environmental Contamination and Toxicology 34(3):248-252.
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: Immobilization
Chemical: o-Dichlorobenzene
HERO ID: 651551

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Test conditions were not reported, only that they followed standard methods. Warne 1996 was cited for maintenance methods.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology addressed or reported the intended outcome of interest-immobilization, but details were lacking in regards to the methodology itself.
	Metric 18: Consistency of Outcome Assessment	Medium	The outcome assessment method for determining immobilization was not reported in great detail.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods seemed suitable for these determinations.
	Metric 22: Reporting of Data	Low	EC50 values were reported in Table 1. However, data for each concentration group were not reported. Control data was also not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: This evaluation is for the D. magna exposure to o-DCB. The EC50 for immobilization was determined after a 48h exposure.

Overall Quality Determination

Uninformative

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.			
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; <i>Daphnia magna</i> ; Juvenile			
Health Outcome:	Reproductive/Teratogenic			
Chemical:	o-Dichlorobenzene			
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.	
	Metric 3: Test Substance Purity	Low	The percent purity was not reported. All test chemicals were reported to be of analytical grade.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	In the results section, the use of controls was mentioned but no details were given.	
	Metric 5: Negative Control Response	Low	The biological response of the negative control group for the test substance was not reported.	
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study reported that tests were carried out in closed systems, but major details regarding test media preparation were not given in the paper.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration details for treatment and control groups were not adequately reported in the paper.	
	Metric 9: Measurement of Test Substance Concentration	Low	It was reported that the exposure concentrations were measured frequently during the long-term test, but measurements were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The reproductive test was conducted for 14 days. This duration is acceptable as per OECD test guidelines.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	There was no direct mention of the number of exposure groups and spacing of exposure levels in the paper other than the test concentrations were approximately in a range 1/30 to 1/2 of the 24h LC50. Based on the number of animals and groupings reported, it can be assumed that there were three test concentrations, which is below the recommended number for standard reproductive test (OECD 202).	
	Metric 12: Testing at or Below Solubility Limit	Low	No details were provided to determine whether exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported, but the test organism was appropriate for evaluating the outcome.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups.	
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Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12(2):253-262.			
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; <i>Daphnia magna</i> ; Juvenile			
Health Outcome:	Reproductive/Teratogenic			
Chemical:	o-Dichlorobenzene			
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 10 organisms per concentration, but no replicates were reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were reported in the paper, but there were not enough details given to determine whether differences occurred between control and exposed populations.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported and did not follow the recommended OECD test guidelines of recording number of eggs in the brood pouch or counting the new born of the F1 generation at an interval of 48-72 hours, etc.	
	Metric 18: Consistency of Outcome Assessment	Low	No details were provided regarding the execution of the study protocol for outcome assessment.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details 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 presented to suggest differences among treatment groups were due to exposure unrelated reasons.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described well.	
	Metric 22: Reporting of Data	Low	The number of young live and the number of young dead on each day of counting in the treatments groups and controls were not reported. Only 14d EC 50 and EC 16 values were reported.	
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.	
Additional Comments:	The number of exposure groups and spacing of exposure levels were not clearly reported. Control response and exposure concentration measurements were not provided, and the data for exposure-related findings were not shown for each treatment and control group.			

Overall Quality Determination**Low**

Study Citation:	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.			
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; <i>Daphnia magna</i> ; IRCHA; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	773229			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The chemical source and analytical verification were not reported.	
	Metric 3: Test Substance Purity	Low	The chemical purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The use of negative controls was reported.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported.	
	Metric 6: Randomized Allocation	Low	The use of random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The media preparation was reported in some detail, but details specific to this test chemical were vague.	
	Metric 8: Consistency of Exposure Administration	Medium	No inconsistencies were reported, but details were sparse. Some test chambers were covered and some were not. Covered chambers were only fed on water renewal days at higher rates, whereas uncovered chambers were fed daily.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Some measurements were reported for the test chemical.	
	Metric 10: Exposure Duration and Frequency	High	The duration was standard and sufficient to assess outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of the groups were not reported as the actual test concentrations were not reported. However, a concentration range was reported.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Extra steps were required to dissolve the chemical. Limited details of the chemical concentrations gave uncertainty to the actual concentration levels.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Organisms were reported to be from in house cultures and were 24h old at the start of the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were reasonably documented.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect.	
Domain 5: Outcome Assessment				

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Study Citation: Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. *Water Research* 23(4):501-510.
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*; IRCHA; Juvenile
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 773229

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Test organism housing, environmental conditions, food, water, and nutrients were appropriate.
	Metric 17: Outcome Assessment Methodology	Medium	The methods of determining chronic effects were reported.
	Metric 18: Consistency of Outcome Assessment	Medium	Limited details were provided, so it was difficult to assess consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Only NOEC values were reported. Statistical methods were reported for the chronic test including the Student's t-test and the U-test.
	Metric 22: Reporting of Data	Low	No raw data was reported for the chronic test. Only NOEC values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation is for the NOEC value reported for the chronic *D. magna* test for o-dichlorobenzene. According to Table 1, parental mortality, reproduction rate, and appearance of first offspring all contributed to the determination of the NOEC value. This form is for the parental mortality (mortality outcome).

Overall Quality Determination

Medium

Study Citation:	Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to <i>Daphnia magna</i> in the 21 day reproduction test. <i>Water Research</i> 23(4):501-510.			
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; <i>Daphnia magna</i> ; IRCHA; Juvenile			
Health Outcome:	Reproductive/Teratogenic			
Chemical:	o-Dichlorobenzene			
HERO ID:	773229			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The chemical source and analytical verification were not reported.	
	Metric 3: Test Substance Purity	Low	The chemical purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The use of negative controls was reported.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported.	
	Metric 6: Randomized Allocation	Low	The use of random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The media preparation was reported in some detail, but details specific to this test chemical were vague.	
	Metric 8: Consistency of Exposure Administration	Medium	No inconsistencies were reported, but details were sparse. Some test chambers were covered and some were not. Covered chambers were only fed on water renewal days at higher rates, whereas uncovered chambers were fed daily.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Some measurements were reported for the test chemical.	
	Metric 10: Exposure Duration and Frequency	High	The duration was standard and sufficient to assess outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups and the spacing of the groups were not reported as the actual test concentrations were not reported. However, a concentration range was reported.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Extra steps were required to dissolve the chemical. Limited details of the chemical concentrations gave uncertainty to the actual concentration levels.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Organisms were reported to be from in house cultures and were 24h old at the start of the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were reasonably documented.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effect.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Test organism housing, environmental conditions, food, water, and nutrients were appropriate.	

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Study Citation: Kühn, R., Pattard, M., Pernak, K. D., Winter, A. (1989). Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. *Water Research* 23(4):501-510.
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*; IRCHA; Juvenile
Health Outcome: Reproductive/Teratogenic
Chemical: o-Dichlorobenzene
HERO ID: 773229

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	The methods of determining chronic effects were reported.
	Metric 18: Consistency of Outcome Assessment	Medium	Limited details were provided, so it was difficult to assess consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Only NOEC values were reported. Statistical methods were reported for the chronic test including the Student's t-test and the U-test.
	Metric 22: Reporting of Data	Low	No raw data was reported for the chronic test. Only NOEC values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: This evaluation is for the NOEC value reported for the chronic *D. magna* test for o-dichlorobenzene. According to Table 1, parental mortality, reproduction rate, and appearance of first offspring all contributed to the determination of the NOEC value. This form is for the reproduction rate and the appearance of the first offspring (reproductive outcome).

Overall Quality Determination

Medium

Study Citation:	Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.
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; <i>Tanytarsus dissimilis</i> ; Larvae
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	3634370; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the 1,2-DCB was reported to be Aldrich Chemical Co. It was not reported if it was analytically verified.
	Metric 3: Test Substance Purity	High	The purity of the 1,2-DCB was reported to be 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a negative control.
	Metric 5: Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported (Table 7).
	Metric 6: Randomized Allocation	Low	It was not reported how the midges were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The test system was reported to be dishes with 10-20 midge larvae in their third or fourth instar. Test solution was dripped into the dishes after an overnight acclimation period. Details regarding test concentration preparation were not reported.
	Metric 8: Consistency of Exposure Administration	Low	Little information was provided on the test exposure. There were 10-20 midges in their third or fourth instar in each test dish. It is unclear if this varied per test chemical or within each dish in the individual exposure.
	Metric 9: Measurement of Test Substance Concentration	Medium	Exposure concentrations were measured using gas-liquid chromatography, the parameters of which are presented in Table C-2. Measured concentrations were reported in Table B-1.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 48h, which is acceptable for an acute midge test.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five reported exposure concentrations (see Table B-1), and the spacing appears adequate for a response.
	Metric 12: Testing at or Below Solubility Limit	High	Measured test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The midges were reported to be from the Environmental Research Laboratory in Duluth, MN USA EPA. The organisms were cultured at the performing laboratory. Larvae in the third or fourth instar were used for this test.
	Metric 14: Acclimatization and Pretreatment Conditions	High	It appears that the midges were cultured in-house and were cultured in conditions similar to those of the test conditions.

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Study Citation: Call, D. J., Brooke, L. T., Ahmad, N., Richter, J. E. (1983). Toxicity and metabolism studies with EPA (Environmental Protection Agency) priority pollutants and related chemicals in freshwater organisms.
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; *Tanytarsus dissimilis*; Larvae
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 3634370; Linked HERO ID(s): 3634370, 7494661

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	There were 10-20 organisms per test chamber, and only two replicates per test concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test and culture conditions were reported and appeared adequate for organism health.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality as reported in LC50 values.
	Metric 18: Consistency of Outcome Assessment	High	Mortality was determined every 24h and appeared to be assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Trimmed Spearman-Kärber method was reported to be used to determine LC50 values.
	Metric 22: Reporting of Data	Low	Data for each test concentration was not reported. Only LC50 values were reported. Table 7 contains LC50 values for midges.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. 95% confidence intervals were reported in Table 7.

Additional Comments: This evaluation is for the acute toxicity of o-DCB on midges. Results are reported in Table 7.

Overall Quality Determination

Medium

Study Citation:	Zhang, S., Lin, D., Wu, F. (2016). The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae. <i>Journal of Hazardous Materials</i> 311:186-193.			
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:	Vegetation; Non-vascular Plants; <i>Chlorella pyrenoidosa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	3607057			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was reported in the supplementary data as Sinopharm Chemical Reagent Co., but it was not reported to be analytically verified.	
	Metric 3: Test Substance Purity	High	The purity from the manufacturer was reported in the supplementary data as 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	The bioaccumulation methods detail that they used the same methods as the toxicity experiments (section 2.2) which has control groups.	
	Metric 5: Negative Control Response	Low	The control algae response was not reported in the bioaccumulation experiment.	
	Metric 6: Randomized Allocation	Low	Allocation methods were not described.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test solution preparation was detailed in the methods, and additional details (algal media composition) were provided in supplementary data.	
	Metric 8: Consistency of Exposure Administration	High	The methods indicate that the groups were sampled and treated the same.	
	Metric 9: Measurement of Test Substance Concentration	Low	The study quantified the test compound in the algal cells, and GC analysis conditions were reported in the supplementary data. Recovery of spiked samples was also reported.	
	Metric 10: Exposure Duration and Frequency	High	Concentrations of the test compound were examined at 4, 8, 12, 24, 48, and 96h which covers the period of the acute bioassay for this taxa.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	The bioaccumulation study looked at one test concentration (1.5 mg/L).	
	Metric 12: Testing at or Below Solubility Limit	High	The test substance was tested below the reported solubility (156 mg/L).	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal culture was reported as the Institute of Wuhan Hydrobiology.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Initial culture conditions were not described.	
	Metric 15: Number of Organisms and Replicates per Group	Low	Replication for this bioassay was poorly described.	
Domain 5: Outcome Assessment				

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Study Citation:	Zhang, S., Lin, D., Wu, F. (2016). The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae. <i>Journal of Hazardous Materials</i> 311:186-193.
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:	Vegetation; Non-vascular Plants; <i>Chlorella pyrenoidosa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	3607057

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	culture conditions (light, temp, initial algae concentration, culture medium) were reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—accumulation of 1,2-DCB in algal cells.
	Metric 18: Consistency of Outcome Assessment	High	Assessment appeared to be consistent among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Details describing the culture conditions during the bioconcentration experiment were not available.
	Metric 20: Outcomes Unrelated to Exposure	Low	No information was presented for this bioaccumulation study.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	T-tests were completed on some metrics for comparisons among groups, however, it does not appear that any statistical analysis was completed on the bioaccumulation data.
	Metric 22: Reporting of Data	Low	A figure representing the bioaccumulation of the test compound are presented in Figure 4B, but control results are not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	The metrics were reported in figure 4B, however, they are very difficult to interpret the values or their variance.

Additional Comments: This study determined the uptake and bioaccumulation of the test compound in algae cells. The larger body of work demonstrated potential differences in organic load of the treatment water while it also demonstrated the role of chloride position and number has on uptake and accumulation. In isolation, the results on the bioaccumulation of the test compound are limiting.

Overall Quality Determination

Medium

Study Citation:	Zhang, S., Lin, D., Wu, F. (2016). The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae. Journal of Hazardous Materials 311:186-193.
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:	Vegetation; Non-vascular Plants; <i>Chlorella pyrenoidosa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	3607057

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was reported in the supplementary data as Sinopharm Chemical Reagent Co., but it was not reported to be analytically verified.
	Metric 3: Test Substance Purity	High	The purity from the manufacturer was reported in the supplementary data as 99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	There was a reported negative control: "the concentration of CB leading to a 50% reduction in algal growth compared with the control."
	Metric 5: Negative Control Response	Low	The control algae growth in 96hr was not reported and the control growth curve reported in Fig S1B was not viewable (supplementary data).
	Metric 6: Randomized Allocation	Low	Allocation methods were not described.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Test solution preparation was detailed in methods, and additional details (algal media composition) were provided in the supplementary data.
	Metric 8: Consistency of Exposure Administration	Medium	The conditions and handling of treatments were similar across groups and the control. Daily administration of the test compound was poorly described as "Supplemental CBs were added every day."
	Metric 9: Measurement of Test Substance Concentration	Low	Although the study quantified the test compound in the algal cells elsewhere in the paper for an uptake experiment, they appear to report the nominal concentrations for this toxicity bioassay and do not report validation of the nominal concentrations.
	Metric 10: Exposure Duration and Frequency	High	This study uses a 96hr duration for algae, which was acceptable for the outcomes of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The study reported a "range" as 0 to 25 mg/L for the test substance but nothing in the methods indicates the spacing of this range.
	Metric 12: Testing at or Below Solubility Limit	High	The test substance was tested below the reported solubility (156 mg/L).
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The source of the algal culture was reported as the Institute of Wuhan Hydrobiology.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The initial culture conditions were not described.
	Metric 15: Number of Organisms and Replicates per Group	Low	Replication for this bioassay was poorly described.
Domain 5: Outcome Assessment			

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Study Citation:	Zhang, S., Lin, D., Wu, F. (2016). The effect of natural organic matter on bioaccumulation and toxicity of chlorobenzenes to green algae. Journal of Hazardous Materials 311:186-193.
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:	Vegetation; Non-vascular Plants; <i>Chlorella pyrenoidosa</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	3607057

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	High	Culture conditions (light, temp, initial algae concentration, culture medium) were reported.
	Metric 17: Outcome Assessment Methodology	Low	The methods for counting algae were detailed, however, details on dilution factors for counting and the number of replicate counts per treatment concentration were not detailed.
	Metric 18: Consistency of Outcome Assessment	High	The assessment appeared to be consistent among treatment concentrations.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Details describing the culture conditions during the bioassay were not available.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No data was provided to indicate any differences among groups was detected.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	The methods for the calculation of 96hr IC50 and NOECs were reported.
	Metric 22: Reporting of Data	Low	Figure 6 displays dose-response curves (log scale for dose), but there is no narrative text to list the NOEC and IC50 concentrations and their error. The methods report that the NOEC was calculated, but it was not apparent in the results or supplementary data.
	Metric 23: Explanation of Unexpected Outcomes	Low	The metrics were reported in figure 6, however, they are very difficult to interpret the values or their variance. The narrative of the results section (3.4) reports no direct concentration values for the test substance.
Additional Comments:	The study shows a dose-response curve for the test compound and supplementary data are helpful in the confidence of the study, however, the results section is poor in informing the reader on the values associated with this curve (fig 6). The paper discusses the formulation of a NOEC value, however, it is not presented in the results section.		

Overall Quality Determination

Medium

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.

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Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5162728

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Chlorella vulgaris* in OECD media.

Overall Quality Determination

High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Chlorella vulgaris*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Chlorella vulgaris* in BBM media.

Overall Quality Determination High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Chlorella vulgaris</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Chlorella vulgaris*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Chlorella vulgaris* in EPA media.

Overall Quality Determination High

Study Citation:	Figueroa, I. D., Simmons, M. S. (1991). Structure-activity relationships of chlorobenzenes using DNA measurement as a toxicity parameter in algae. <i>Environmental Toxicology and Chemistry</i> 10(3):323-330.		
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:	Vegetation; Non-vascular Plants; <i>Cyclotella meneghiniana</i> ; Strain CyOH2; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-Genotox (including DNA repair)		
Chemical:	o-Dichlorobenzene		
HERO ID:	659965		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The source was identified but wasn't analytically verified.
	Metric 3: Test Substance Purity	Medium	The test substance was identified as reagent grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls were used.
	Metric 5: Negative Control Response	Medium	Control response was reported in the text, but no data was shown.
	Metric 6: Randomized Allocation	Medium	Randomized allocation was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Some experimental system information was reported but not in much detail.
	Metric 8: Consistency of Exposure Administration	High	No inconsistencies were reported.
	Metric 9: Measurement of Test Substance Concentration	High	Test concentrations were measured.
	Metric 10: Exposure Duration and Frequency	High	A standard assay duration was used.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	A good range of concentrations were tested.
	Metric 12: Testing at or Below Solubility Limit	High	Stock preparation was documented, and seemed adequate.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	Standard characteristics of algal cultures were followed and reported.
	Metric 14: Acclimatization and Pretreatment Conditions	High	A standard acclimatization protocol was followed.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Adequate cell numbers were used.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Standard test conditions were followed and presented.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.

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Study Citation: Figueroa, I. D., Simmons, M. S. (1991). Structure-activity relationships of chlorobenzenes using DNA measurement as a toxicity parameter in algae. *Environmental Toxicology and Chemistry* 10(3):323-330.
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: Vegetation; Non-vascular Plants; *Cyclotella meneghiniana*; Strain CyOH2; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mechanistic-Genotox (including DNA repair)
Chemical: o-Dichlorobenzene
HERO ID: 659965

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reasonably well documented.
	Metric 22: Reporting of Data	Medium	Data for exposure-related findings were presented for each treatment group and were adequate to determine values for the endpoint of interest. Control data was lumped into the EC determinations, but no raw control data was shown.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination

High

Study Citation:	Kühn, R., Pattard, M. (1990). Results of the harmful effects of water pollutants to green algae (<i>Scenedesmus subspicatus</i>) in the cell multiplication inhibition test. <i>Water Research</i> 24(1):31-38.			
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:	Vegetation; Non-vascular Plants; <i>Scenedesmus subspicatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	1023091			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The chemical source and analytical verification were not reported.	
	Metric 3: Test Substance Purity	Low	The test chemical purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were used.	
	Metric 5: Negative Control Response	High	Population growth for controls was reported at several time intervals.	
	Metric 6: Randomized Allocation	Low	Random allocation was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of the exposure administration were reported and adequate.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Test chemical concentrations were measured but not reported.	
	Metric 10: Exposure Duration and Frequency	High	Standard test durations were used.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	A range of test concentrations was provided, but the actual test concentrations were not reported.	
	Metric 12: Testing at or Below Solubility Limit	Medium	Solubility was based on optical clarity.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Acclimatization procedures were adequately described.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Algal housing, environmental conditions, food, water, and nutrients were conducive to the maintenance of the culture.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment was reported in adequate detail.	
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Study Citation: Kühn, R., Pattard, M. (1990). Results of the harmful effects of water pollutants to green algae (*Scenedesmus subspicatus*) in the cell multiplication inhibition test. *Water Research* 24(1):31-38.
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: Vegetation; Non-vascular Plants; *Scenedesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 1023091

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	No confounding variables were reported as altering the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis of data was adequate.
	Metric 22: Reporting of Data	Low	Only ECx values were reported. Data for each exposure concentration was not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	The difference in toxicity of the chlorinated benzenes was accounted for.

Additional Comments: This evaluation is for the effect of o-dichlorobenzene on the inhibition of growth of *S. subspicatus*.

Overall Quality Determination High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Scenedesmus subspicatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Scenedesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Scenedesmus subspicatus* in BBM media.

Overall Quality Determination

High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Scenedesmus subspicatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Scenedesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Scenedesmus subspicatus* in OECD media.

Overall Quality Determination High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.			
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Scenedesmus subspicatus</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5162728			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.	
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.	
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.	

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Scenedesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Scenedesmus subspicatus* in EPA media.

Overall Quality Determination

High

Study Citation:	Galassi, S., Vighi, M. (1981). Testing toxicity of volatile substances with algae. Chemosphere 10(10):1123-1126.		
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:	Vegetation; Non-vascular Plants; <i>Selenastram capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	1990600; Linked HERO ID(s): 4930936, 1990600		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	Neither source nor analytical verification of chemical identity were reported.
	Metric 3: Test Substance Purity	Low	The chemical purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative control use was reported.
	Metric 5: Negative Control Response	Medium	Results were expressed as a percentage of the growth in the control culture, but no specific control results were given.
	Metric 6: Randomized Allocation	Low	Authors did not report how the algae were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Medium	Concentrations were measured but not initially due to volatility.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was standard for algal growth inhibition tests.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Test concentrations covered a wide range, and they provided a suitable dose response.
	Metric 12: Testing at or Below Solubility Limit	Medium	There was a process to making the test concentrations. Stock solutions were prepared by adding an amount of chemical ten times higher than the saturation solubility to distilled water in a closed vessel, that was stirred for 48 hours and decanted for 24 hours. The supernatant was filtered and the concentration measured. Final solutions were made by adding different amounts of stock solution to 10 mL of stock culture medium. Initial concentrations in the medium were too difficult to measure, so nominal test concentrations were calculated from the dilution of the titrated stock solutions.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	There was little information given on the test algae used.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Author cited AAPBT methods, but few details were reported.
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of replicates was not reported.

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Study Citation:	Galassi, S., Vighi, M. (1981). Testing toxicity of volatile substances with algae. Chemosphere 10(10):1123-1126.
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:	Vegetation; Non-vascular Plants; <i>Selenastram capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1990600; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Authors cited following AAPBT methods. However, not a lot of details regarding test conditions were given.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported and seemed consistent.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	There were no reported differences among the study groups in environmental conditions, but few details were provided on acclimation or test conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	EC50 values were graphically determined which seems somewhat inadequate, and no information was given on what statistical methods were performed, if any.
	Metric 22: Reporting of Data	Low	Raw data was not reported.
	Metric 23: Explanation of Unexpected Outcomes	Medium	There were minor uncertainties regarding how test concentrations were identified, and the results were compared to literature values.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.		
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.
	Metric 3: Test Substance Purity	Medium	Percent purity was not reported, but the test compounds were reported to be analytical grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	Low	Minimal details were given in the paper with respect to a control. There were two cited references for methodology. One was unavailable. The available reference (HERO ID: 1990600) mentions a control in the results section but does not give any details.
	Metric 5: Negative Control Response	Low	Biological response of the negative control group was not reported.
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	There were two cited references for methodology. One was unavailable. The available reference provided some details of test media preparation but lacked key details.
	Metric 8: Consistency of Exposure Administration	Low	There were two cited references for methodology. One was unavailable. The paper and the available reference did not provide details of exposure administration.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured but not reported in the paper. In one of the available cited references (HERO ID: 1990600), nominal concentration at the start of the experiment and mean concentrations based on the measurements after equilibration and during the test were reported. Nominal concentrations and measured concentrations were very different.
	Metric 10: Exposure Duration and Frequency	High	This was an algal assay with a 96h IGC50 (inhibition growth concentration for 50% of the population).
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups and the spacing of exposure levels in the paper. However, in one of the available cited references, a table of nominal concentrations was reported, but these concentrations were different from the measured concentrations reported in the same reference.
	Metric 12: Testing at or Below Solubility Limit	High	Per one of the cited references, test concentrations were below the solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.

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Study Citation: Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere* 12(2):253-262.
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: Vegetation; Non-vascular Plants; *Selenastrum capricornutum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups.
	Metric 15: Number of Organisms and Replicates per Group	Low	The starting algal concentration was 5 x 10 ⁶ cells/l, but no replicates were reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	The measurement of pH and dissolved oxygen were mentioned in the paper but lacked sufficient details.
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported.
	Metric 18: Consistency of Outcome Assessment	Low	No details were provided regarding the execution of the study protocol for outcome assessment.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details 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 presented to suggest differences among treatment groups were due to unrelated reasons.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described well.
	Metric 22: Reporting of Data	Low	Data for exposure related findings were not reported. Only 96h EC 50 values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments: The number of exposure groups, exposure concentrations (nominal or measured), spacing of exposure levels, and control response were not reported. Only 96h EC 50 values were reported.			

Overall Quality Determination

Uninformative

Study Citation:	Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. <i>Chemosphere</i> 12(2):253-262.
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Photosynthesis
Chemical:	o-Dichlorobenzene
HERO ID:	4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source was not reported, and the test substance identity was not analytically verified.
	Metric 3: Test Substance Purity	Low	Percent purity was not reported. All test chemicals were reported to be of analytical grade.
Domain 2: Test Design			
	Metric 4: Negative Controls	Uninformative	The use of controls was not reported in the paper.
	Metric 5: Negative Control Response	Low	The biological response of the negative control group for the test substance was not reported.
	Metric 6: Randomized Allocation	Low	There was no report on how the test organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	Adequate details regarding test media preparation were not given in the paper.
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration details for treatment and control groups were not adequately reported in the paper.
	Metric 9: Measurement of Test Substance Concentration	Low	It is reported that the exposure concentrations were measured, but measurements were not reported.
	Metric 10: Exposure Duration and Frequency	High	The 3h EC50 test for photosynthesis was acceptable for determining the outcome of interest. The authors were able to derive EC 50 values.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The number of exposure groups and the spacing levels were not reported.
	Metric 12: Testing at or Below Solubility Limit	Low	No details were provided to determine whether exposure concentrations exceeded the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported, but the test organism was appropriate for evaluating the outcome.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and whether pretreatment conditions were the same for control and exposed groups.
	Metric 15: Number of Organisms and Replicates per Group	Medium	Algal biomass corresponding to 0.1 mg dry weight was used and tests were carried out in triplicate.
Domain 5: Outcome Assessment			

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Study Citation: Calamari, D., Galassi, S., Setti, F., Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere* 12(2):253-262.
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: Vegetation; Non-vascular Plants; *Selenastrum capricornutum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mechanistic-Photosynthesis
Chemical: o-Dichlorobenzene
HERO ID: 4930936; Linked HERO ID(s): 4930936, 1990600

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were reported in the paper, but not enough details given to determine whether differences occurred between control and exposed populations.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology was reported.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was conducted after 3 hours.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The paper does not provide any details 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 presented to suggest differences among treatment groups were due to exposure unrelated reasons.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described well.
	Metric 22: Reporting of Data	Low	Data for each treatment and control groups were not reported. Only EC50 values were reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.
Additional Comments:	The exposure concentrations (nominal or measured), spacing of exposure levels, the use of controls, and the control response were not reported. Only 3h EC 50 values were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Cassery, D. M., Davis, E. M., Downs, T. D., Guthrie, R. K. (1983). Sorption of organics by <i>Selenastrum capricornutum</i> . Water Research 17(11):1591-1594.
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1333375

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	Neither the source nor analytical verification were reported.
	Metric 3: Test Substance Purity	Low	Chemical purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	Low	The control response was not reported, but for a BCF study it would be assumed to be a blank.
	Metric 6: Randomized Allocation	Low	The study did not report that organisms were randomly allocated.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	Medium	It was reported that a colonial type bacterium was found in the algae culture and could not be eliminated for test initiation. Study authors reported that an indeterminate but small amount of sorption may have been presumed to have been bacterial.
	Metric 9: Measurement of Test Substance Concentration	Medium	Chemical concentrations in the test media and the algae were measured.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was 24h. This appeared adequate for the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one test concentration as seen in Table 1, as the goal of the study was to assess BCF values, not to observe a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	The author makes a point of addressing this for the media employed. The test concentration was below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The algae was reported to be from the National Eutrophication Research Program, Pacific Northwest Environmental Research Laboratory, National Environmental Research Center, U.S.E.P.A in Corvallis, OR. The algae was reported to be in the log growth phase at test initiation.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms although details were scant.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were two replicates reported, but the initial cell count was not reported.

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Study Citation:	Casserly, D. M., Davis, E. M., Downs, T. D., Guthrie, R. K. (1983). Sorption of organics by <i>Selenastrum capricornutum</i> . Water Research 17(11):1591-1594.
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1333375

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	The algae were cultured in 3 gallon bottles with modified Bold's Basal Medium with TRIS buffer. The photoperiod was reported to be 14L:10D, but little other information as provided.
	Metric 17: Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed or reported the intended outcomes of interest. Study authors reported that algal biomass was measured as TSS. It is possible that something else in the medium could contribute to the value.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	The effects of the bacterial contamination were unknown.
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors reported bacterial contamination in the algal cultures. The effects of the contamination are unknown.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	A linear model was used for data analysis which seems appropriate.
	Metric 22: Reporting of Data	High	TSS was reported in Table 1. TSS was used as a biomass measurement.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported. Values seem to correlate with Log Kow.
Additional Comments:	This evaluation is for the TSS values. TSS was reported to be a measurement of biomass for the algae, which would indicate a development/growth outcome.		

Overall Quality Determination

Medium

Study Citation:	Casserly, D. M., Davis, E. M., Downs, T. D., Guthrie, R. K. (1983). Sorption of organics by <i>Selenastrum capricornutum</i> . Water Research 17(11):1591-1594.
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1333375

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	Neither the source nor analytical verification were reported.
	Metric 3: Test Substance Purity	Low	Chemical purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	Low	The control response was not reported, but for a BCF study it would be assumed to be a blank.
	Metric 6: Randomized Allocation	Low	The study did not report that organisms were randomly allocated.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	Medium	It was reported that a colonial type bacterium was found in the algae culture and could not be eliminated for test initiation. Study authors reported that an indeterminate but small amount of sorption may have been presumed to have been bacterial.
	Metric 9: Measurement of Test Substance Concentration	Medium	Chemical concentrations in the test media and the algae were measured.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was 24h. This appeared adequate for the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one test concentration as seen in Table 1, as the goal of the study was to assess BCF values, not to observe a dose response.
	Metric 12: Testing at or Below Solubility Limit	High	The author makes a point of addressing this for the media employed. The test concentration was below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The algae was reported to be from the National Eutrophication Research Program, Pacific Northwest Environmental Research Laboratory, National Environmental Research Center, U.S.E.P.A in Corvallis, OR. The algae was reported to be in the log growth phase at test initiation.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	The test organisms were acclimatized to test conditions and all pretreatment conditions were the same for control and exposed organisms although details were scant.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were two replicates reported, but the initial cell count was not reported.
Domain 5: Outcome Assessment			

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Study Citation:	Casserly, D. M., Davis, E. M., Downs, T. D., Guthrie, R. K. (1983). Sorption of organics by <i>Selenastrum capricornutum</i> . Water Research 17(11):1591-1594.
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:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1333375

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	The algae were cultured in 3 gallon bottles with modified Bold's Basal Medium with TRIS buffer. The photoperiod was reported to be 14L:10D, but little other information as provided.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-BCF values.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes appeared to be assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	The effects of the bacterial contamination were unknown.
	Metric 20: Outcomes Unrelated to Exposure	Low	Authors reported bacterial contamination in the algal cultures. The effects of the contamination are unknown.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	A linear model was used for data analysis which seems appropriate.
	Metric 22: Reporting of Data	High	BCFs were reported as were absorption summaries.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported. Values seem to correlate with Log Kow.

Additional Comments: This evaluation is for the sorption of o-DCB in the algal species *S. capricornutum*.

Overall Quality Determination

Medium

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. <i>Water Research</i> 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Selenastrum capricornutum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Selenastrum capricornutum* in EPA media.

Overall Quality Determination

High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Selenastrum capricornutum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Selenastrum capricornutum* in OECD media.

Overall Quality Determination

High

Study Citation:	Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. Water Research 22(12):1593-1597.		
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5162728		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	The purity and/or grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported using an appropriate concurrent negative control group.
	Metric 5: Negative Control Response	High	The biological responses of the negative control group was adequate and reported in Figure 1.
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of the test media were described in adequate detail.
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and the spacing of exposure levels were adequate to address the purpose of the study.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were at or below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The test organisms were adequately described and were obtained from a reliable source.
	Metric 14: Acclimatization and Pretreatment Conditions	High	All pretreatment conditions were the same for control and exposed organisms.
	Metric 15: Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were reported and sufficient to characterize toxicological effects.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Contents of culture media were reported. The culture and test temperature was reported to be 22C, and they were shaken at 160 rpm under constant illumination.
	Metric 17: Outcome Assessment Methodology	High	The algae was counted every 24h via an electronic particle counter. Growth of the culture was the intended outcome of interest.

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Study Citation: Millington, L. A., Goulding, K. H., Adams, N. (1988). The influence of growth medium composition on the toxicity of chemicals to algae. *Water Research* 22(12):1593-1597.
Duration: Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path: Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Vegetation; Non-vascular Plants; *Selenastrum capricornutum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5162728

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in terms of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were clearly described.
	Metric 22: Reporting of Data	Medium	Only the results for the lowest concentration of test chemicals causing significant reduction in growth in the three algal media were reported (Table 3). Control results were reported in Figure 1.
	Metric 23: Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for the growth of *Selenastrum capricornutum* in BBM media.

Overall Quality Determination

High

Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.			
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:	Vegetation; Vascular Plants; <i>Bacopa caroliniana</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1998227			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was reported but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Percent purity reported as 98.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Study authors reported using analytical blanks.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured using appropriate analytical technologies and methods, but they were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type and outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one concentration per study type, as a dose response was not the intended outcome.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentration was well below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The test organisms were adequately described. However, they were reported to be obtained from the wild, which creates concern regarding organisms health.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was unclear if the test organisms were acclimatized prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	0.5gsh of plant samples about 5-10cm long were exposed during the study, but the number of organisms per chamber and the number of replicates were not clear.	
Domain 5: Outcome Assessment				
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Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. Environmental Toxicology and Chemistry 10(5):665-680.
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:	Vegetation; Vascular Plants; <i>Bacopa caroliniana</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1998227

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Little information was provided on the maintenance or storage of the plants prior to the start of the study. Only temperature and photoperiod were reported for the test conditions. Water quality and characteristics were not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were appropriate for the dataset.
	Metric 22: Reporting of Data	Medium	Time course accumulation was reported in some detail, but control responses were not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation was for the uptake of o-DCB by fresh *Bacopa caroliniana* shoots. Comparisons were also made with frozen samples.

Overall Quality Determination

Medium

Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.			
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:	Vegetation; Vascular Plants; <i>Hydrilla verticillata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1998227			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was reported but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Percent purity reported as 98.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Study authors reported using analytical blanks.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured using appropriate analytical technologies and methods, but they were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type and outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one concentration per study type, as a dose response was not the intended outcome.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentration was well below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The test organisms were adequately described. However, they were reported to be obtained from the wild, which creates concern regarding organisms health.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was unclear if the test organisms were acclimatized prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	0.5gsh of plant samples about 5-10cm long were exposed during the study, but the number of organisms per chamber and the number of replicates were not clear.	
Domain 5: Outcome Assessment				

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Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. Environmental Toxicology and Chemistry 10(5):665-680.
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:	Vegetation; Vascular Plants; <i>Hydrilla verticillata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1998227

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Little information was provided on the maintenance or storage of the plants prior to the start of the study. Only temperature and photoperiod were reported for the test conditions. Water quality and characteristics were not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were appropriate for the dataset.
	Metric 22: Reporting of Data	Medium	Time course accumulation was reported in some detail, but control responses were not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation was for the uptake of o-DCB by fresh *Hydrilla verticillata* shoots. Comparisons were also made with frozen samples.

Overall Quality Determination

Medium

Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. <i>Environmental Toxicology and Chemistry</i> 10(5):665-680.			
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:	Vegetation; Vascular Plants; <i>Myriophyllum spicatum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1998227			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was reported but the test substance identity was not analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	Percent purity reported as 98.6%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	Study authors reported using analytical blanks.	
	Metric 5: Negative Control Response	Low	No control response was reported.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The experimental system and methods for preparation of test media were described in adequate detail.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported, and exposures were administered consistently across study groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were measured using appropriate analytical technologies and methods, but they were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type and outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one concentration per study type, as a dose response was not the intended outcome.	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentration was well below the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The test organisms were adequately described. However, they were reported to be obtained from the wild, which creates concern regarding organisms health.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was unclear if the test organisms were acclimatized prior to the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Low	0.5gsh of plant samples about 5-10cm long were exposed during the study, but the number of organisms per chamber and the number of replicates were not clear.	
Domain 5: Outcome Assessment				
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Study Citation:	Wolf, S. D., Lassiter, R. R., Wooten, S. E. (1991). Predicting chemical accumulation in shoots of aquatic plants. Environmental Toxicology and Chemistry 10(5):665-680.
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:	Vegetation; Vascular Plants; <i>Myriophyllum spicatum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1998227

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Little information was provided on the maintenance or storage of the plants prior to the start of the study. Only temperature and photoperiod were reported for the test conditions. Water quality and characteristics were not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	No inconsistencies were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were appropriate for the dataset.
	Metric 22: Reporting of Data	Medium	Time course accumulation was reported in some detail, but control responses were not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Unexpected outcomes were satisfactorily explained.

Additional Comments: This evaluation was for the uptake of o-DCB by fresh *Myriophyllum spicatum* shoots. Comparisons were also made with frozen samples.

Overall Quality Determination

Medium

Study Citation:	Yoshioka, Y., Ose, Y., Sato, T. (1985). Testing for the toxicity of chemicals with <i>Tetrahymena pyriformis</i> . <i>Science of the Total Environment</i> 43(1-2):149-157.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Other; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Tetrahymena pyriformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	676758		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The chemical source and analytical verification were not reported.
	Metric 3: Test Substance Purity	Low	The chemical purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A blank was used as the control. Population growth was assessed for the blank.
	Metric 5: Negative Control Response	Medium	Test conditions affected the control response.
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	High	Authors followed conditions number 4 for culturing the organisms. These conditions were described adequately.
	Metric 8: Consistency of Exposure Administration	Low	It was difficult to determine if exposures were administered consistently across study groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Test concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The test duration was adequate to assess the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of exposure groups was not reported. Only EC50 values were reported.
	Metric 12: Testing at or Below Solubility Limit	Medium	Exposure range was not reported but authors reported that if solubility was an issue, they would use DMSO to help solubilize the test substance.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	Minimal information reported for test organism. It was uncertain if the test organism was cultured at the performing laboratory.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Minimal information was reported for test organism acclimatization.
	Metric 15: Number of Organisms and Replicates per Group	Low	The initial number of organisms used for the toxicity test was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Procedure number 4 was followed, but details were limited.

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Study Citation:	Yoshioka, Y., Ose, Y., Sato, T. (1985). Testing for the toxicity of chemicals with <i>Tetrahymena pyriformis</i> . Science of the Total Environment 43(1-2):149-157.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Other; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Tetrahymena pyriformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	676758		
Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	Two different methods were used to count final numbers. Then, the two numbers were averaged.
	Metric 18: Consistency of Outcome Assessment	Medium	No inconsistencies were reported, but details were not well documented.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Variables such as temperature and media were discussed, but they weren't mentioned regarding frequency of occurrence or deviation from protocol.
	Metric 20: Outcomes Unrelated to Exposure	Medium	Variables such as contamination were discussed, but the frequencies and occurrence weren't reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Population growth rate was plotted on graph paper, and the EC50 value was determined from this.
	Metric 22: Reporting of Data	Low	Only EC50 values were reported. Results for each test concentration were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Medium	Variables such as contamination were discussed but weren't mentioned regarding frequency of occurrence.
Additional Comments:	This evaluation was on the effect of 1,2-DCB on the population growth of the protozoa, <i>T. pyriformis</i> . Development/growth was selected as the outcome of interest.		

Overall Quality Determination

Low

Study Citation:	Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows (<i>Cyprinodon variegatus</i>). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.			
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:	Vertebrate; Fish; <i>Cyprinodon variegatus</i> ; Juvenile			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	18110; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	All that was reported was that the test chemicals were from chemical supply companies.	
	Metric 3: Test Substance Purity	Low	It was reported that all test chemicals had a purity of 80% or greater, but the actual purity for the chemical of interest was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control.	
	Metric 5: Negative Control Response	Low	The control response was not reported. Only LC50 values were reported.	
	Metric 6: Randomized Allocation	Low	It was not reported how the fish were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Very little information was provided on the preparation of the test substance. Tests were reported to be static. It was unclear if appropriate steps were taken to prevent any volatilization of the test substance.	
	Metric 8: Consistency of Exposure Administration	Low	Little information was provided on the test system, so consistency is hard to determine. Two types of test chambers were used. It is unclear which type was used for each test chemical. Only nominal concentrations were used, and test concentrations were not analyzed, so exposure consistency across the test duration cannot be determined.	
	Metric 9: Measurement of Test Substance Concentration	Low	Study authors did not report measuring the test concentrations.	
	Metric 10: Exposure Duration and Frequency	High	The 96h exposure duration is typical for an acute toxicity test with fish.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	The test concentrations were not reported, nor was the number of exposure groups.	
	Metric 12: Testing at or Below Solubility Limit	Low	The test concentrations were not reported, so it cannot be determined if they were under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The sheepshead minnows were reported to be from the BMRL laboratory or from the EPA Gulf Breeze laboratory. Juvenile minnows were reported to be used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the fish were acclimated to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported there were 10 fish per test chamber, but the number of replicates was not reported.	

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Study Citation: Heitmuller, P. T., Hollister, T. A., Parrish, P. R. (1981). Acute toxicity of 54 industrial chemicals to sheepshead minnows (*Cyprinodon variegatus*). Bulletin of Environmental Contamination and Toxicology 27(5):596-604.
Duration: Overall 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: Vertebrate; Fish; *Cyprinodon variegatus*; Juvenile
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 18110; Linked HERO ID(s): 7508, 18050, 18064, 18110, 628983

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Holding conditions were reported for the fish, but little detail was provided regarding test conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality in the form of LC50 values.
	Metric 18: Consistency of Outcome Assessment	High	Mortality was assessed every 24h for the duration of the study.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Study authors reported using three statistical methods depending on the nature of the data: moving average angle analysis, probit analysis, or binomial probability.
	Metric 22: Reporting of Data	Low	Only LC50 values and NOEC values were reported. Data for each exposure level and control were not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. 95% confidence intervals were reported in Table I.
Additional Comments:	This evaluation was for the acute toxicity of o-DCB on the <i>C. variegatus</i> . The 96h study received an unacceptable rating due to lack of information regarding the test concentrations used and the spacing of the exposure concentrations.		

Overall Quality Determination

Uninformative

Study Citation:	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). <i>Bulletin of Environmental Contamination and Toxicology</i> 54(1):36-42.			
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:	Vertebrate; Fish; <i>Solea solea</i> ; L.; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	5253054			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test 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	AnalaR grade was used with purity between 97-99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative and solvent controls were used in this study.	
	Metric 5: Negative Control Response	Low	No control results were reported, just LC50 values with 95% confidence intervals.	
	Metric 6: Randomized Allocation	Low	No indication of randomized allocation was reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	This was a semi-static test with renewals every 48h. The test substance preparation was reported. Distilled acetone was used as a solvent, and dissolution was further optimized via sonication. Test concentrations were reported to be within 80% of the nominal values.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentration measurements were not reported, though it was reported that test concentrations were measured and remained within 80% of the nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and was appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was reported there was five experimental exposure groups, but the test concentrations were not reported, so spacing was unclear.	
	Metric 12: Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	There are reservations about the source of test organisms. The test organisms were wild caught, so the health of the organisms was uncertain. The age was not reported, though test organisms of similar sized were used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number for an acute test.	

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Study Citation:	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). <i>Bulletin of Environmental Contamination and Toxicology</i> 54(1):36-42.
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:	Vertebrate; Fish; <i>Solea solea</i> ; L.; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5253054

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently reported to evaluate if they were adequate.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Study authors reported that LC50 values were calculated using probit analysis.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation was for the LC50 values estimated for *Solea solea* after exposure to o-DCB. This study received an unacceptable rating due to lack of information regarding the test concentrations used and the spacing of the concentrations.

Overall Quality Determination

Uninformative

Study Citation:	Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. <i>Aquatic Toxicology</i> 8(3):163-174.			
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; <i>Artemia</i> ; Larvae			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	1486051			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source was not reported.	
	Metric 3: Test Substance Purity	High	Test substance purity was reported as at least 97%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control group was reported.	
	Metric 5: Negative Control Response	High	Control group mortality was less than 10%, as reported in the text.	
	Metric 6: Randomized Allocation	Low	The paper does not report on how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	Test media preparation methods were briefly given in this paper, and another reference was cited, which was reviewed. Appropriate measures were taken to minimize loss of test substances.	
	Metric 8: Consistency of Exposure Administration	High	The study cites another reference, which was reviewed. Exposure administration was similar among groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, and nominal concentrations of the treatment groups were not reported.	
	Metric 10: Exposure Duration and Frequency	High	This was an acute <i>Artemia</i> study with a 24-hr duration.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was reported that at least 5 exposure groups were tested, but no information was provided on the exposure concentrations or the spacing of exposure levels.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the solubility limit. It was reported that a saturated aqueous stock solution was prepared and then diluted to provide 5 exposure concentrations.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	Test organisms of similar age (early instar II nauplii) were chosen as per the paper and the cited reference. The source of the <i>Artemia</i> cysts was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Per the cited reference, nauplii were held in sea water at 20C for 26 hours, but it does not report whether pretreatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The cited reference reported the use of 10 animals per vial and 2-4 replicates per test concentration.	

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Study Citation: Abernethy, S., Bobra, A. M., Shiu, W. Y., Wells, P. G., Mackay, D. (1986). Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to two planktonic crustaceans: The key role of organism-water partitioning. *Aquatic Toxicology* 8(3):163-174.

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; *Artemia*; Larvae

Health Outcome: Mortality

Chemical: o-Dichlorobenzene

HERO ID: 1486051

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions were not reported other than temperature. Loading biomass exceeds (10 animals per 33 ml vial) the recommendation in the EPA Series 850 Ecological Effects Test Guidelines.
Metric 17:	Outcome Assessment Methodology	High	The mortality assessment was conducted at 24 h and 48 h using a Stereostar zoom microscope, but only LC50 values for 24 h were reported.
Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was done at 24 h and appears to be consistent across study groups.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest outcomes unrelated to exposure was reported.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical analysis was conducted. LC50 values, slope factors, and 95% confidence limits were calculated by the graphical method of Litchfield and Wilcoxon (1948).
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. Only LC 50 values were presented.
Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: The study did not provide nominal or measured concentrations of treatment groups and did not provide dose-response data. Only LC50 values were reported. This form is for the acute toxicity of o-DCB to *Artemia*.

Overall Quality Determination

Uninformative

Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . <i>Chemosphere</i> 80(1):20-27.		
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; <i>Melita longidactyla</i> ; Adult		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	1002318		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance (Sigma, Australia) was reported, but it was not analytically verified.
	Metric 3: Test Substance Purity	Medium	It is mentioned that AR grade is used but does not provide the percentage purity.
Domain 2: Test Design			
	Metric 4: Negative Controls	Uninformative	A control group was not reported.
	Metric 5: Negative Control Response	Low	The biological response of the negative control group was not reported.
	Metric 6: Randomized Allocation	Low	The study does not report randomized allocation.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The methods of preparation of test media are given but no measures were taken to minimize the loss of test substance before and during the exposure. The concentrations of the test substance were not measured before or during the study.
	Metric 8: Consistency of Exposure Administration	Medium	The solvent concentration was not reported.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured at the beginning or at the end of the test.
	Metric 10: Exposure Duration and Frequency	High	Exposure duration was for 48 hours for the acute toxicity test.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	11 exposure groups were reported and allowed for the identification of an LC50 value.
	Metric 12: Testing at or Below Solubility Limit	Low	A solvent concentration was not reported, and a solvent control was not used.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The size and the source of the animals were reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Animals were acclimatized in seawater for a month, but no control group was reported.
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 10 amphipods in each replicate, and there were 3 replicates per concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions before the test were reported ,but environmental/housing conditions during the test were not reported. No control group was reported.

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Study Citation: Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, *Melita longidactyla*. *Chemosphere* 80(1):20-27.
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; *Melita longidactyla*; Adult
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 1002318

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	Acute toxicity test methodology was appropriate for the mortality outcome assessment.
	Metric 18: Consistency of Outcome Assessment	High	The outcome assessment was conducted at regular intervals.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions during the test were not reported.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No information was presented to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Acute toxicity test data was not reported. It was mentioned that the authors used probit analysis to find LC50 value.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group. The LC50 value was presented in the text, but no raw data was reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report mortality data or any measures of variability.

Additional Comments: The acute toxicity test in this study did not include control groups, and environmental conditions and mortality data for exposure groups were not reported.

Overall Quality Determination

Uninformative

Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . <i>Chemosphere</i> 80(1):20-27.			
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	1002318			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance (Sigma, Australia) was reported, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Medium	It is mentioned that AR grade is used, but it does not provide the percentage purity.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (solvent and seawater controls) were used.	
	Metric 5: Negative Control Response	High	Negative control responses were adequate.	
	Metric 6: Randomized Allocation	Low	Authors do not report how organisms were allocated to treatment groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Test media preparation details were reported, and the renewal of media was done daily. The paper mentions preliminary studies conducted to find the dissipation of the test substance from the test solution, but the amount of solvent used as a carrier was not reported, and the concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	The same protocol was used for all exposure groups, however; the study does not provide details of a solvent concentration used in the exposure groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported.	
	Metric 10: Exposure Duration and Frequency	High	Sub-lethal tests were carried out for 5 weeks.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Three exposure concentrations (well below the measured LC50 values) were used and were adequate to obtain results for a growth outcome.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the solubility limit of the test substance.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The size and the source of the test organisms were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Test organisms were acclimatized in seawater for one month before being used for experiments, but it does not report whether pretreatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 9 replicates per treatment and control groups, and 10 organisms per replicate were used.	

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Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . Chemosphere 80(1):20-27.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1002318

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions before the test were reported, but the environmental conditions during the 5 week test were not reported. Therefore, one cannot assess whether differences occurred between control and exposed populations.
Metric 17:	Outcome Assessment Methodology	High	Growth assessment was done by measuring biomass, RNA:DNA ratio and scope for growth.
Metric 18:	Consistency of Outcome Assessment	High	Growth assessment protocol was similar among treatment groups and controls.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	No information was presented on environmental conditions (temperature, pH, dissolved oxygen, etc.) of the exposure or control groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest growth differences among groups unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods used are presented clearly.
Metric 22:	Reporting of Data	High	Data for each treatment and control group were reported.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Sub-lethal tests to assess growth outcome (via biomass and scope of growth) were conducted with adequate replicates and negative control groups. Data and statistical analysis were presented clearly but environmental conditions during the test were not reported.		

Overall Quality Determination

Medium

Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . Chemosphere 80(1):20-27.			
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult			
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Developmental and juvenile toxicology			
Chemical:	o-Dichlorobenzene			
HERO ID:	1002318			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance (Sigma, Australia) was reported, but it was not analytically verified.	
	Metric 3: Test Substance Purity	Medium	It is mentioned that AR grade is used, but it does not provide the percentage purity.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls (solvent and seawater controls) were used.	
	Metric 5: Negative Control Response	High	Negative control responses were adequate.	
	Metric 6: Randomized Allocation	Low	Authors do not report how organisms were allocated to treatment groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Test media preparation details were reported, and the renewal of media was done daily. The paper mentions preliminary studies conducted to find the dissipation of the test substance from the test solution, but the amount of solvent used as a carrier was not reported, and the concentrations of the test substance were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	The same protocol was used for all exposure groups, however; the study does not provide details of a solvent concentration used in the exposure groups.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported.	
	Metric 10: Exposure Duration and Frequency	High	Sub-lethal tests were carried out for 5 weeks.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Three exposure concentrations (well below the measured LC50 values) were used and were adequate to obtain results for a growth outcome.	
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the solubility limit of the test substance.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The size and the source of the test organisms were reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Test organisms were acclimatized in seawater for one month before being used for experiments, but it does not report whether pretreatment conditions were the same for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 9 replicates per treatment and control groups, and 10 organisms per replicate were used.	
Domain 5: Outcome Assessment				
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Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . Chemosphere 80(1):20-27.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Developmental and juvenile toxicology
Chemical:	o-Dichlorobenzene
HERO ID:	1002318

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions before the test were reported, but the environmental conditions during the 5 week test were not reported. Therefore, one cannot assess whether differences occurred between control and exposed populations.
	Metric 17: Outcome Assessment Methodology	High	Growth assessment was done by measuring biomass, RNA:DNA ratio and scope for growth.
	Metric 18: Consistency of Outcome Assessment	High	Growth assessment protocol was similar among treatment groups and controls.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	No information was presented on environmental conditions (temperature, pH, dissolved oxygen, etc.) of the exposure or control groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest growth differences among groups unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods used are presented clearly.
	Metric 22: Reporting of Data	High	Data for each treatment and control group were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: Sub-lethal tests to assess growth outcome (DNA:RNA ratio) were conducted with adequate replicates and negative control groups. Data and statistical analysis were presented clearly but environmental conditions during the test were not reported.

Overall Quality Determination

Medium

Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . Chemosphere 80(1):20-27.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	o-Dichlorobenzene
HERO ID:	1002318

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance (Sigma, Australia) was reported, but it was not analytically verified.
	Metric 3: Test Substance Purity	Medium	It is mentioned that AR grade is used, but it does not provide the percentage purity.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Negative controls (solvent and seawater controls) were used.
	Metric 5: Negative Control Response	High	Negative control responses were adequate.
	Metric 6: Randomized Allocation	Low	Authors do not report how organisms were allocated to treatment groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test media preparation details were reported, and renewal of media was done daily. The paper mentions preliminary studies conducted to find the dissipation of the test substance from the test solution, but the amount of solvent used as a carrier was not reported, and the concentrations of the test substance were not measured during the study.
	Metric 8: Consistency of Exposure Administration	Medium	The same protocol was used for all exposure groups, however; the study does not provide details of the solvent concentration used in the exposure groups.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported.
	Metric 10: Exposure Duration and Frequency	High	Two reproductive end points (percentage of females producing progeny and number of progeny per female) were measured after 21 days of exposure, and total fecundity were determined after 60 days of exposure. The paper reports conducting preliminary experiments to identify the appropriate duration of exposure.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Two exposure concentrations (well below the measured LC50 values) were used and were adequate to obtain results of reproductive outcome.
	Metric 12: Testing at or Below Solubility Limit	High	Exposure concentrations were below the solubility limit of the test substance.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	High	The size and source of the test organisms were reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Test organisms were acclimatized in seawater for one month before being used for experiments, but it does not report whether pretreatment conditions were the same for control and exposed groups.
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 3 replicates per treatment and control group, and 10 organisms per replicate were used.

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Study Citation:	Tong, E. S., Merwe, J. P., Chiu, J. M., Wu, R. S. (2010). Effects of 1,2-dichlorobenzene on the growth, bioenergetics and reproduction of the amphipod, <i>Melita longidactyla</i> . Chemosphere 80(1):20-27.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 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; <i>Melita longidactyla</i> ; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	o-Dichlorobenzene
HERO ID:	1002318

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions during the 21 d and 60 d of exposure were not reported. Therefore, one cannot assess whether differences occurred between control and exposed populations.
Metric 17:	Outcome Assessment Methodology	High	Reproductive outcome assessment methodology was appropriate. Two reproductive end points (percentage of females producing progeny and number of progeny per female) were measured after 21 days of exposure, and total fecundity was determined after 60 days of exposure.
Metric 18:	Consistency of Outcome Assessment	High	Reproductive outcome assessment protocol was similar among treatment groups and controls. The newly emerged juveniles were counted daily and removed from each experimental vessel.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	No information was presented on environmental conditions.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in reproductive outcome unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods used are presented clearly.
Metric 22:	Reporting of Data	High	Data for each treatment and control groups were reported.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.
Additional Comments:	Sub-lethal tests to assess reproductive outcomes were conducted with adequate replicates and negative control groups. Data and statistical analysis were presented clearly, but environmental conditions during the test were not reported.		

Overall Quality Determination

Medium

Study Citation:	Curtis, M., Ward, C. (1981). Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. Journal of Hydrology 51(1):359-367.		
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; <i>Palaemon pugio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	5352394; Linked HERO ID(s): 1392282, 5352394		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substances was not reported.
	Metric 3: Test Substance Purity	Low	The purity of the test substance was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of appropriate concurrent negative controls.
	Metric 5: Negative Control Response	High	It was reported that control mortality was less than 10% in every case.
	Metric 6: Randomized Allocation	Low	It was not reported how organisms were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	Some toxicants were added directly to the test tanks, and in other cases, a stock solution was added to the test tanks. The preparation of the stock solutions was not reported, and it was not reported which chemicals were added directly to the test tanks and which had stock solutions prepared. All tests were reported to be static tests.
	Metric 8: Consistency of Exposure Administration	High	All bioassay test chambers were filled with 12 L of dilution water to a depth of approximately 24cm. A circulating bath was used to maintain a temp of 22C.
	Metric 9: Measurement of Test Substance Concentration	Low	Water samples were taken at the beginning and the end of each test to analyze for toxicant concentration. Electron capture gas chromatography was used to analyze the test concentrations. Actual test measurements were not reported, but relative error and relative standard deviation were reported in Table I. Study authors report that results of the toxicant analyses were primarily used for physiochemical behaviors of the toxicants, not for computing LC50 values.
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be 96 hours, which is acceptable for an acute toxicity study with shrimp.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported that for each chemical at least five test concentrations were tested in a 0.6 geometric series. The exact test concentrations were not reported.
	Metric 12: Testing at or Below Solubility Limit	Low	Exact test concentrations were not reported, so it is unclear if any of the test concentrations were above the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	It was reported that the grass shrimp were collected from wild populations in an estuary near Galveston Bay, Texas. The age of the test organisms was not reported.

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Study Citation:	Curtis, M., Ward, C. (1981). Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. Journal of Hydrology 51(1):359-367.		
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; <i>Palaemon pugio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	5352394; Linked HERO ID(s): 1392282, 5352394		
Domain	Metric	Rating	Comments
	Metric 14: Acclimatization and Pretreatment Conditions	High	The grass shrimp were observed for 10 days before use in definitive testing under the same conditions as those used during exposure.
	Metric 15: Number of Organisms and Replicates per Group	Low	There were only five test organisms per test chamber and two replicates per treatment concentration.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Test organisms were held at 22C at a pH range of 8.3-8.7. Conductivity was reported, but little else regarding environmental conditions was reported. It was not reported if or what the shrimp were fed prior to the start of the test. Photoperiod was not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality in terms of LC50 values.
	Metric 18: Consistency of Outcome Assessment	High	For the duration of the study, deaths were recorded and bodies were removed whenever observed. Every 24 hours, the number of surviving organisms was determined.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not report sufficient details of environmental conditions to allow for a comparison.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was conducted using one of three methods depending on the data obtained. These methods included probit, moving average, or binomial test method depending on the number of partial kills in each test. The exact method for the shrimp o-dichlorobenzene test was not reported. Also, it was reported that confidence intervals represented the range of sensitivities of the organisms rather than the precision of the acute mortality tests.
	Metric 22: Reporting of Data	Low	Continuous data for each treatment level was not presented. Only LC50 values were reported. Confidence intervals were reported as a range of sensitivities rather than precision of the tests.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability in these tests was represented as a range of sensitivities in the organisms. The highest concentration to produce zero mortality and the lowest concentration to produce 100% mortality were used as conservative 95% confidence limits. These ranges were not reflective of the precision of the acute mortality test.

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Study Citation:	Curtis, M., Ward, C. (1981). Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. Journal of Hydrology 51(1):359-367.
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; <i>Palaemon pugio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5352394; Linked HERO ID(s): 1392282, 5352394

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the acute toxicity of o-dichlorobenzene to grass shrimp. Mortality was the outcome of interest. LC50 values were reported, but little other data was provided. 95% confidence intervals were reported represent ranges of sensitivities rather than precision of the test. The highest concentration to produce zero mortality and the lowest concentration to produce 100% mortality were used as conservative 95% confidence limits.		

Overall Quality Determination	Low
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Study Citation:	Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. <i>Water Research</i> 13(2):137-141.			
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; <i>Palaemonetes pugio</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	1392282; Linked HERO ID(s): 1392282, 5352394			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	Source was not reported, and the chemical identity not analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity of the test substance was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were used.	
	Metric 5: Negative Control Response	High	Mortality in the controls was less than 10%, as reported in the text.	
	Metric 6: Randomized Allocation	Low	The study does not report random allocation of test organisms.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study does not provide sufficient details of test media preparation for testing the compound of interest.	
	Metric 8: Consistency of Exposure Administration	Uninformative	In the results section, it is stated that o-dichlorobenzene was administered in crystal form and remained undissolved.	
	Metric 9: Measurement of Test Substance Concentration	Low	Nominal concentrations and exposure concentrations (though measured) were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The 96 hr acute toxicity test was appropriate for the outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	No information was provided on the number of exposure groups.	
	Metric 12: Testing at or Below Solubility Limit	Low	Nominal and exposure concentrations were not reported and cannot determine whether the exposure concentration exceeded the solubility limit. It was reported that the test chemical was not fully dissolved and it was "visibly insoluble at the ranges tested."	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	It was reported that the test organisms were wild caught from an estuary in the Galveston Bay system. This creates concern regarding the initial health of the organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The test organisms were acclimatized but it cannot be determined if the pretreatment conditions were the same for the control and the exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 5 animals per replicate and 2 replicates per concentration.	
Domain 5: Outcome Assessment				

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Study Citation: Curtis, M. W., Copeland, T. L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. *Water Research* 13(2):137-141.

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; *Palaemonetes pugio*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mortality

Chemical: o-Dichlorobenzene

HERO ID: 1392282; Linked HERO ID(s): 1392282, 5352394

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	Environmental conditions were recorded during the test but results were not reported. Temperature was reported to be 22C in both test waters. The reconstituted dilution water characteristics were reported as well. Organisms were fed during holding and not fed for the test, which is typical. DO and temperature were monitored daily for the test.
	Metric 17: Outcome Assessment Methodology	High	The mortality outcome assessment was appropriate for the test.
	Metric 18: Consistency of Outcome Assessment	Medium	Mortality was checked at 24, 48, and 96 hours, but does not provide details to assess whether this protocol was consistent for control and treatment groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study does not report the data on environmental conditions recorded during the test.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences in the outcome unrelated to the exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	LC 50 values and the confidence intervals were determined using probit analysis.
	Metric 22: Reporting of Data	Low	No data was reported on nominal or exposure concentrations, mortality outcomes for each concentration, or environmental conditions. Only LC 50 values were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.
Additional Comments:	This evaluation is for o-DCB in saltwater. The study did not report the exposure or nominal concentrations, the number of exposure groups, environmental conditions the of controls and the treatment groups during the test, or the dose-response data. Only LC 50 values were reported.		

Overall Quality Determination

Uninformative

Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.		
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; sperm exposure; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-Cytotoxicity		
Chemical:	o-Dichlorobenzene		
HERO ID:	1003942		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The test chemicals were purchased from Aldrich in Milwaukee, WI, but they were not reported to be analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	The percent purity was reported as $\geq 99\%$.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Both negative and solvent controls were used in the study.
	Metric 5: Negative Control Response	High	The negative control responses for the blank and solvent control were reported in Tables 2 and 3.
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations or on the test system.
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions are likely to have an impact on the results. Too little information was provided to assess if the administration was consistent.
	Metric 9: Measurement of Test Substance Concentration	Low	Chemical concentrations were not measured. Only nominal concentrations were used.
	Metric 10: Exposure Duration and Frequency	High	Sperm exposure was for 5-90 minutes or for 30 minutes depending on what was being assessed in the embryo development. This appeared adequate to observe a response.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one test level was used, as the goal was not to have a dose response but to observe for developmental and cytogenetic effects in embryos fertilized with sperm exposed to the COI.
	Metric 12: Testing at or Below Solubility Limit	Medium	DMSO was used as a solvent to alleviate any solubility concerns.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The organisms were reported to be caught from the Bay of Naples in the Mediterranean Sea. This creates concern regarding the health of the organisms.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated.
	Metric 15: Number of Organisms and Replicates per Group	Low	There was no indication of the number of organisms tested.

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Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; sperm exposure; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Cytotoxicity
Chemical:	o-Dichlorobenzene
HERO ID:	1003942

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	General test conditions were reported, but few details were provided.
	Metric 17: Outcome Assessment Methodology	Medium	Few details were provided on how the outcomes were assessed. Frequencies of developmental defects involving skeletal and/or gut malformations were observed, but how these were determined is unclear. Little information was provided on how cytogenetic effects were assessed either.
	Metric 18: Consistency of Outcome Assessment	Medium	Few details were provided on the outcome assessment methodology, so it was difficult to assess the consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported outcomes unrelated to the study groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reported and seemed suitable.
	Metric 22: Reporting of Data	High	Results for control and treatment responses were reported in Tables 2 and 3 for sperm treated with the COI and then used to fertilize embryos.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes, and variability was reported in Table 1.
Additional Comments:	This evaluation was for the exposure of o-DCB to sea urchin sperm. Treated sperm were then used to fertilize untreated embryos and cytogenetic effects in the F1 embryos were assessed.		

Overall Quality Determination

Medium

Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; sperm exposure; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Reproductive/Teratogenic
Chemical:	o-Dichlorobenzene
HERO ID:	1003942

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.
	Metric 2: Test Substance Source	Low	The test chemicals were purchased from Aldrich in Milwaukee, WI, but they were not reported to be analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	High	The percent purity was reported as $\geq 99\%$.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Both negative and solvent controls were used in the study.
	Metric 5: Negative Control Response	High	The negative control responses for the blank and solvent control were reported in Tables 2 and 3.
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations or on the test system.
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions are likely to have an impact on the results. Too little information was provided to assess if the administration was consistent.
	Metric 9: Measurement of Test Substance Concentration	Low	Chemical concentrations were not measured. Only nominal concentrations were used.
	Metric 10: Exposure Duration and Frequency	High	Sperm exposure was for 5-90 minutes or for 30 minutes depending on what was being assessed in the embryo development. This appeared adequate to observe a response.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one test level was used, as the goal was not to have a dose response but to observe for developmental and cytogenetic effects in embryos fertilized with sperm exposed to the COI.
	Metric 12: Testing at or Below Solubility Limit	Medium	DMSO was used as a solvent to alleviate any solubility concerns.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The organisms were reported to be caught from the Bay of Naples in the Mediterranean Sea. This creates concern regarding the health of the organisms.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated.
	Metric 15: Number of Organisms and Replicates per Group	Low	There was no indication of the number of organisms tested.
Domain 5: Outcome Assessment			

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Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; sperm exposure; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Reproductive/Teratogenic
Chemical:	o-Dichlorobenzene
HERO ID:	1003942

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Medium	General test conditions were reported, but few details were provided.
	Metric 17: Outcome Assessment Methodology	Medium	Few details were provided on how the outcomes were assessed. Frequencies of developmental defects involving skeletal and/or gut malformations were observed, but how these were determined is unclear. Little information was provided on how cytogenetic effects were assessed either.
	Metric 18: Consistency of Outcome Assessment	Medium	Few details were provided on the outcome assessment methodology, so it was difficult to assess the consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported outcomes unrelated to the study groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reported and seemed suitable.
	Metric 22: Reporting of Data	High	Results for control and treatment responses were reported in Tables 2 and 3 for sperm treated with the COI and then used to fertilize embryos.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes, and variability was reported in Table 1.

Additional Comments: This evaluation was for the exposure of o-DCB to sea urchin sperm. Treated sperm were then used to fertilize untreated embryos and developmental and cytogenetic effects in the embryos were assessed.

Overall Quality Determination

Medium

Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.			
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; Embryo			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	1003942			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test chemicals were purchased from Aldrich in Milwaukee, WI, but they were not reported to be analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The percent purity was reported as $\geq 99\%$.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both negative and solvent controls were used in the study.	
	Metric 5: Negative Control Response	High	The negative control responses for the blank and solvent control were reported in Table 1.	
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations or on the test system.	
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions are likely to have an impact on the results. Too little information was provided to assess if the administration was consistent.	
	Metric 9: Measurement of Test Substance Concentration	Low	Chemical concentrations were not measured. Only nominal concentrations were used.	
	Metric 10: Exposure Duration and Frequency	High	Embryo exposure was for 48h. This appeared to be adequate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only three exposure concentrations, which is lower than is typical. However, the spacing appears adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	DMSO was used as a solvent to alleviate any solubility concerns.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The organisms were reported to be caught from the Bay of Naples in the Mediterranean Sea. This creates concern regarding the health of the organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There was no indication of the number of organisms tested.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	General test conditions were reported, but few details were provided.	

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Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; Embryo
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	1003942

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	Few details were provided on how the outcomes were assessed. Frequencies of developmental defects involving skeletal and/or gut malformations were observed, but how these were determined is unclear.
	Metric 18: Consistency of Outcome Assessment	Medium	Few details were provided on the outcome assessment methodology, so it was difficult to assess the consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported outcomes unrelated to the study groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reported and seemed suitable.
	Metric 22: Reporting of Data	High	Developmental defects for all exposure concentrations and the controls were reported in Table 1.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes, and variability was reported in Table 1.

Additional Comments: This evaluation was for the exposure of o-DCB to sea urchin embryos to observe developmental defects. It was reported that skeletal and gut malformations were assessed, but little information was provided on how these were assessed.

Overall Quality Determination **Medium**

Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.			
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; Embryo			
Health Outcome:	Mechanistic-Cytotoxicity			
Chemical:	o-Dichlorobenzene			
HERO ID:	1003942			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test chemicals were purchased from Aldrich in Milwaukee, WI, but they were not reported to be analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The percent purity was reported as $\geq 99\%$.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both negative and solvent controls were used in the study.	
	Metric 5: Negative Control Response	High	The negative control responses for the blank and solvent control were reported in Table 1.	
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The study provided only limited details on the measures taken to appropriately prepare test concentrations or on the test system.	
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions are likely to have an impact on the results. Too little information was provided to assess if the administration was consistent.	
	Metric 9: Measurement of Test Substance Concentration	Low	Chemical concentrations were not measured. Only nominal concentrations were used.	
	Metric 10: Exposure Duration and Frequency	High	Embryo exposure was for 48h. This appeared to be adequate for the outcomes of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only three exposure concentrations, which is lower than is typical. However, the spacing appears adequate for a dose response.	
	Metric 12: Testing at or Below Solubility Limit	Medium	DMSO was used as a solvent to alleviate any solubility concerns.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The organisms were reported to be caught from the Bay of Naples in the Mediterranean Sea. This creates concern regarding the health of the organisms.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Low	There was no indication of the number of organisms tested.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	General test conditions were reported, but few details were provided.	
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Study Citation:	Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Giordano, G. G., Ragucci, E., Trieff, N. M. (1988). Comparative toxicities of benzene, chlorobenzene, and dichlorobenzenes to sea urchin embryos and sperm. <i>Bulletin of Environmental Contamination and Toxicology</i> 40(4):481-488.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Paracentrotus lividus</i> ; Embryo
Health Outcome:	Mechanistic-Cytotoxicity
Chemical:	o-Dichlorobenzene
HERO ID:	1003942

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Medium	Few details were provided on how the outcomes were assessed. Mitotic abnormalities, including total mitotic aberrations, mean number mitoses per embryo, % interphase embryos, metaphase/anaphase ratio, and % anaphase aberrations were assessed, but little information was provided on how they were assessed.
	Metric 18: Consistency of Outcome Assessment	Medium	Few details were provided on the outcome assessment methodology, so it was difficult to assess the consistency.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported outcomes unrelated to the study groups, but few details were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods were reported and seemed suitable.
	Metric 22: Reporting of Data	High	Cytogenetic effects for all exposure concentrations and the controls were reported in Table 1.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes, and variability was reported in Table 1.

Additional Comments: This evaluation was for the exposure of o-DCB to sea urchin embryos to observe cytogenetic effects. Details on how these effects were assessed were limited.

Overall Quality Determination

Medium

Study Citation:	Oshida, P. S. (1977). Toxicity of a chlorinated benzene to sea urchin embryos.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); <i>Strongylocentrotus purpuratus</i> ; Embryo
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5255526

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test chemical was only identified by name.
	Metric 2: Test Substance Source	Low	The source was not reported and the test substance identity was not analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	Low	The test substance purity was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	The use of a negative control was reported.
	Metric 5: Negative Control Response	High	The negative control response was reported and adequate.
	Metric 6: Randomized Allocation	Low	Authors did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	The experimental system and test media preparation methods were adequately reported, but did not completely account for the physical-chemical properties of the test substance.
	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	Authors reported measuring test solutions, but the measurement methods were not indicated. Also, measured test concentrations at the end of the exposure were not reported.
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and appropriate for the study type.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	Only two test concentrations were used.
	Metric 12: Testing at or Below Solubility Limit	Low	There was an issue with the solubility of the liquid o-DCB being added to seawater. The solution had to be stirred continuously for the duration of the experiment.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Medium	The test organisms were collected intertidally from Lunada Bay on the Palos Verdes Peninsula.
	Metric 14: Acclimatization and Pretreatment Conditions	High	The sea urchins were acclimated for a week in the laboratory flow-through seawater system at 12 degrees Celcius.
	Metric 15: Number of Organisms and Replicates per Group	Low	Replicates were not used.
Domain 5: Outcome Assessment			

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Study Citation: Oshida, P. S. (1977). Toxicity of a chlorinated benzene to sea urchin embryos.
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; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); *Strongylocentrotus purpuratus*; Embryo
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5255526

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Test conditions during the exposure were not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome.
	Metric 18: Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Study authors reported 0.4 mg/liter of o-DCB in the control after 48 hours.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in health outcomes unrelated to the exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Statistical analysis was not conducted.
	Metric 22: Reporting of Data	Medium	Figures 2 and 3 showed control and treatment results. For the treatment results it is unclear which group they belonged to.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.

Additional Comments: None

Overall Quality Determination

Uninformative

Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.			
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5243980			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test substance source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was reported that pre-cultures with methanol and without test substances were used as the controls. It was reported that pre-cultures were incubated without chemical until the algal biomass reached 1mg/L, and then the test substances were added. It is unclear if the control value was taken before the addition of chemicals to the other flasks, or if the the pre-cultures without test substance were allowed to continue for the duration of the study.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	The study did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system and/or test media preparation methods were not reported in great detail. The DCB was reported to be prepared by adding 1mL of methanol DCB stock solution to a bottle of double distilled water. Stoppers were used to minimize volatilization. Test concentrations were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Test concentrations were not measured, so it is unclear if they remained similar for the duration of the study. Test volumes were not reported. Otherwise, consistency seems adequate.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified 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	High	The test organisms were adequately described and were obtained from a reliable source.	

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Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5243980

Domain	Metric	Rating	Comments
	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. Pre-culture biomass reached about 1mg/L before test substances were added to initiate the tests.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Minor uncertainties regarding salinity were identified regarding organism housing, environmental conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.

Additional Comments: In this evaluation, growth and growth inhibition were assessed in marine algae species *Chlorella marine*.

Overall Quality Determination

Medium

Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.			
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1982535			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The DCB was not certified or analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors reported that there was a control for the preculture portion of the study for TeCB only. Nothing was reported as a control for the other test chemicals, including o-DCB.	
	Metric 5: Negative Control Response	Uninformative	The negative control response was not reported as it was unclear if there was a negative control for chemicals other than TeCB.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was a static system with glass stoppers to minimize volatilization. However, exposure concentrations were unclear, and it did not appear that test concentrations were measured at any point.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure concentrations did not appear to be measured, so it is unclear if test concentrations remained consistent for the entire exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test solutions were made. Analytical measurements were not reported. This was a BCF study.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency were appropriate for the test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations was given, but the spacing was not reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The range of test concentrations was under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal species was reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pre-treatment environmental conditions were well described and equal across groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The biomass of algae was appropriate for algae study, but the number of replicates was not reported.	

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Study Citation: Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. *Chemosphere* 35(8):1781-1797.

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

Health Outcome: ADME (biotransformation)

Chemical: o-Dichlorobenzene

HERO ID: 1982535

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17: Outcome Assessment Methodology	Uninformative	The acute toxicity test used to validate the BCF model. While BCF was reported, determination of toxicity was not.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables were consistent for all groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Results were used to fit the BCF model. Statistics or results for acute toxicity were not reported.
	Metric 22: Reporting of Data	Uninformative	Reported data were not sufficient to determine a hazard endpoint.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation is for the BCF of *Chlorella marine* to o-DCB.

Overall Quality Determination

Uninformative

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiu rong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.		
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	5257521		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The o-DCB was identified by name only.
Metric 2:	Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
Metric 5:	Negative Control Response	Low	The negative control response was not reported. BCFD values were reported.
Metric 6:	Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
Metric 8:	Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This appeared adequate to observe BCFD values.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. BCFD values were reported in Table 1, but little was provided on methods of measuring the test chemical.
	Metric 18: Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Calculations for BCFD values were described, but it was not reported how measurements were taken.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods and equations for BCFD were described.
	Metric 22: Reporting of Data	Low	BCFD values were reported in Table 1, but no control responses were reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on BCFD of the marine algae *Chlorella marine*.

Overall Quality Determination **Low**

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.			
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5257521			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
Metric 1:	Test Substance Identity	Low	The o-DCB was identified by name only.	
Metric 2:	Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.	
Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.	
Metric 5:	Negative Control Response	Medium	The negative control response was reported in Fig.1 and appeared adequate. However, the figure was a bit difficult to read.	
Metric 6:	Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.	
Domain 3: Exposure Characterization				
Metric 7:	Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.	
Metric 8:	Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.	
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.	
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is typical of algae acute toxicity tests.	
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.	
Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.	
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.	
Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.	

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Chlorella marine</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—algal growth in terms of biomass as seen in Fig. 1.
Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Methods for obtaining biomass were not described in detail other than to say at which time points observations were made.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods and equations for growth were described.
Metric 22:	Reporting of Data	Low	Growth of the marine algae in terms of biomass is presented in Fig. 1. EC10, EC50, EC70, and EC90 values are reported in Fig. 6. Data for each test concentration and control were not reported.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on growth of the marine algae *Chlorella marine*.

Overall Quality Determination

Medium

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.		
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:	Vegetation; Non-vascular Plants; <i>Nannochloris oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	5257521		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The o-DCB was identified by name only.
Metric 2:	Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
Metric 5:	Negative Control Response	Low	The negative control response was not reported. BCFD values were reported.
Metric 6:	Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
Metric 8:	Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This appeared adequate to observe BCFD values.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.			
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:	Vegetation; Non-vascular Plants; <i>Nannochloris oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	5257521			
Domain	Metric	Rating	Comments	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.	
	Metric 17: Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. BCFD values were reported in Table 1, but little was provided on methods of measuring the test chemical.	
	Metric 18: Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Calculations for BCFD values were described, but it was not reported how measurements were taken.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods and equations for BCFD were described.	
	Metric 22: Reporting of Data	Low	BCFD values were reported in Table 1, but no control responses were reported.	
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.	
Additional Comments:	This evaluation was for the effect of o-DCB on BCFD of the marine algae <i>Nannochloris oculata</i> .			
Overall Quality Determination		Low		

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Nannochloris oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The o-DCB was identified by name only.
Metric 2:	Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
Metric 5:	Negative Control Response	Medium	The negative control response was reported in Fig.1 and appeared adequate. However, the figure was a bit difficult to read.
Metric 6:	Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
Metric 8:	Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is typical of algae acute toxicity tests.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
Metric 12:	Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	Low	The source of the test organisms was not reported.
Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.
Metric 15:	Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Nannochloris oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—algal growth in terms of biomass as seen in Fig. 1.
Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Methods for obtaining biomass were not described in detail other than to say at which time points observations were made.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods and equations for growth were described.
Metric 22:	Reporting of Data	Low	Growth of the marine algae in terms of biomass is presented in Fig. 1. EC10, EC50, EC70, and EC90 values are reported in Fig. 6. Data for each test concentration and control were not reported.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on growth of the marine algae *Nannochloris oculata*.

Overall Quality Determination

Medium

Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.			
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:	Vegetation; Non-vascular Plants; <i>Nannochloropsis oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5243980			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test substance source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was reported that pre-cultures with methanol and without test substances were used as the controls. It was reported that pre-cultures were incubated without chemical until the algal biomass reached 1mg/L, and then the test substances were added. It is unclear if the control value was taken before the addition of chemicals to the other flasks, or if the the pre-cultures without test substance were allowed to continue for the duration of the study.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	The study did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system and/or test media preparation methods were not reported in great detail. The DCB was reported to be prepared by adding 1mL of methanol DCB stock solution to a bottle of double distilled water. Stoppers were used to minimize volatilization. Test concentrations were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Test concentrations were not measured, so it is unclear if they remained similar for the duration of the study. Test volumes were not reported. Otherwise, consistency seems adequate.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified 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	High	The test organisms were adequately described and were obtained from a reliable source.	

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Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.
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:	Vegetation; Non-vascular Plants; <i>Nannochloropsis oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5243980

Domain	Metric	Rating	Comments
	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. Pre-culture biomass reached about 1mg/L before test substances were added to initiate the tests.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Minor uncertainties regarding salinity were identified regarding organism housing, environmental conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.

Additional Comments: In this evaluation, growth and growth inhibition were assessed in marine algae species *Nannochloropsis oculata*.

Overall Quality Determination

Medium

Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.			
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:	Vegetation; Non-vascular Plants; <i>Nannochloropsis oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1982535			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The DCB was not certified or analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors reported that there was a control for the preculture portion of the study for TeCB only. Nothing was reported as a control for the other test chemicals, including o-DCB.	
	Metric 5: Negative Control Response	Uninformative	The negative control response was not reported as it was unclear if there was a negative control for chemicals other than TeCB.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was a static system with glass stoppers to minimize volatilization. However, exposure concentrations were unclear, and it did not appear that test concentrations were measured at any point.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure concentrations did not appear to be measured, so it is unclear if test concentrations remained consistent for the entire exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test solutions were made. Analytical measurements were not reported. This was a BCF study.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency were appropriate for the test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations was given, but the spacing was not reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The range of test concentrations was under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal species was reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pre-treatment environmental conditions were well described and equal across groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The biomass of algae was appropriate for algae study, but the number of replicates was not reported.	

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Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Nannochloropsis oculata</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1982535

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17: Outcome Assessment Methodology	Uninformative	The acute toxicity test used to validate the BCF model. While BCF was reported, determination of toxicity was not.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables were consistent for all groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Results were used to fit the BCF model. Statistics or results for acute toxicity were not reported.
	Metric 22: Reporting of Data	Uninformative	Reported data were not sufficient to determine a hazard endpoint.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation is for the BCF of *Nannochloropsis oculata* to o-DCB.

Overall Quality Determination

Uninformative

Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.			
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5243980			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test substance source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was reported that pre-cultures with methanol and without test substances were used as the controls. It was reported that pre-cultures were incubated without chemical until the algal biomass reached 1mg/L, and then the test substances were added. It is unclear if the control value was taken before the addition of chemicals to the other flasks, or if the the pre-cultures without test substance were allowed to continue for the duration of the study.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	The study did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system and/or test media preparation methods were not reported in great detail. The DCB was reported to be prepared by adding 1mL of methanol DCB stock solution to a bottle of double distilled water. Stoppers were used to minimize volatilization. Test concentrations were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Test concentrations were not measured, so it is unclear if they remained similar for the duration of the study. Test volumes were not reported. Otherwise, consistency seems adequate.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified 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	High	The test organisms were adequately described and were obtained from a reliable source.	

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Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5243980

Domain	Metric	Rating	Comments
	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. Pre-culture biomass reached about 1mg/L before test substances were added to initiate the tests.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Minor uncertainties regarding salinity were identified regarding organism housing, environmental conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.

Additional Comments: In this evaluation, growth and growth inhibition were assessed in marine algae species *Phaeodactylum tricornutum*.

Overall Quality Determination

Medium

Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.			
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricornutum</i> ; Bohlin; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1982535			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The DCB was not certified or analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors reported that there was a control for the preculture portion of the study for TeCB only. Nothing was reported as a control for the other test chemicals, including o-DCB.	
	Metric 5: Negative Control Response	Uninformative	The negative control response was not reported as it was unclear if there was a negative control for chemicals other than TeCB.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was a static system with glass stoppers to minimize volatilization. However, exposure concentrations were unclear, and it did not appear that test concentrations were measured at any point.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure concentrations did not appear to be measured, so it is unclear if test concentrations remained consistent for the entire exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test solutions were made. Analytical measurements were not reported. This was a BCF study.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency were appropriate for the test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations was given, but the spacing was not reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The range of test concentrations was under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal species was reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pre-treatment environmental conditions were well described and equal across groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The biomass of algae was appropriate for algae study, but the number of replicates was not reported.	

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Study Citation: Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. *Chemosphere* 35(8):1781-1797.

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: Vegetation; Non-vascular Plants; *Phaeodactylum tricornutum*; Bohlin; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: ADME (biotransformation)

Chemical: o-Dichlorobenzene

HERO ID: 1982535

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17: Outcome Assessment Methodology	Uninformative	The acute toxicity test used to validate the BCF model. While BCF was reported, determination of toxicity was not.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables were consistent for all groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Results were used to fit the BCF model. Statistics or results for acute toxicity were not reported.
	Metric 22: Reporting of Data	Uninformative	Reported data were not sufficient to determine a hazard endpoint.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation is for the BCF of *Phaeodactylum tricornutum*-Bohlin to o-DCB.

Overall Quality Determination

Uninformative

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiuorong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. Journal of Ocean University of Qingdao 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
	Metric 5: Negative Control Response	Medium	The negative control response was reported in Fig.1 and appeared adequate. However, the figure was a bit difficult to read.
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is typical of algae acute toxicity tests.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricorutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—algal growth in terms of biomass as seen in Fig. 1.
	Metric 18: Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Methods for obtaining biomass were not described in detail other than to say at which time points observations were made.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods and equations for growth were described.
	Metric 22: Reporting of Data	Low	Growth of the marine algae in terms of biomass is presented in Fig. 1. EC10, EC50, EC70, and EC90 values are reported in Fig. 6. Data for each test concentration and control were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on growth of the marine algae *Phaeodactylum tricorutum*.

Overall Quality Determination**Medium**

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.			
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricorutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	5257521			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported. BCFD values were reported.	
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This appeared adequate to observe BCFD values.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.	
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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Phaeodactylum tricorutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. BCFD values were reported in Table 1, but little was provided on methods of measuring the test chemical.
Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Calculations for BCFD values were described, but it was not reported how measurements were taken.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods and equations for BCFD were described.
Metric 22:	Reporting of Data	Low	BCFD values were reported in Table 1, but no control responses were reported.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on BCFD of the marine algae *Phaeodactylum tricorutum*.

Overall Quality Determination

Low

Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.			
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5243980			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test substance source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was reported that pre-cultures with methanol and without test substances were used as the controls. It was reported that pre-cultures were incubated without chemical until the algal biomass reached 1mg/L, and then the test substances were added. It is unclear if the control value was taken before the addition of chemicals to the other flasks, or if the the pre-cultures without test substance were allowed to continue for the duration of the study.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	The study did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system and/or test media preparation methods were not reported in great detail. The DCB was reported to be prepared by adding 1mL of methanol DCB stock solution to a bottle of double distilled water. Stoppers were used to minimize volatilization. Test concentrations were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Test concentrations were not measured, so it is unclear if they remained similar for the duration of the study. Test volumes were not reported. Otherwise, consistency seems adequate.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified 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	High	The test organisms were adequately described and were obtained from a reliable source.	

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Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5243980

Domain	Metric	Rating	Comments
	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. Pre-culture biomass reached about 1mg/L before test substances were added to initiate the tests.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Minor uncertainties regarding salinity were identified regarding organism housing, environmental conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: In this evaluation, growth and growth inhibition were assessed in marine algae species <i>Platymonas subcordiformis</i> .			

Overall Quality Determination**Medium**

Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.			
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1982535			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The DCB was not certified or analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors reported that there was a control for the preculture portion of the study for TeCB only. Nothing was reported as a control for the other test chemicals, including o-DCB.	
	Metric 5: Negative Control Response	Uninformative	The negative control response was not reported as it was unclear if there was a negative control for chemicals other than TeCB.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was a static system with glass stoppers to minimize volatilization. However, exposure concentrations were unclear, and it did not appear that test concentrations were measured at any point.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure concentrations did not appear to be measured, so it is unclear if test concentrations remained consistent for the entire exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test solutions were made. Analytical measurements were not reported. This was a BCF study.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency were appropriate for the test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations was given, but the spacing was not reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The range of test concentrations was under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal species was reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pre-treatment environmental conditions were well described and equal across groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The biomass of algae was appropriate for algae study, but the number of replicates was not reported.	

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Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. Chemosphere 35(8):1781-1797.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route,	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Media, Path:	
Taxa, Species, Age:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1982535

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17: Outcome Assessment Methodology	Uninformative	The acute toxicity test used to validate the BCF model. While BCF was reported, determination of toxicity was not.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables were consistent for all groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Results were used to fit the BCF model. Statistics or results for acute toxicity were not reported.
	Metric 22: Reporting of Data	Uninformative	Reported data were not sufficient to determine a hazard endpoint.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation is for the BCF of *Platymonas subcordiformis* to o-DCB.

Overall Quality Determination

Uninformative

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiuorong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
	Metric 5: Negative Control Response	Medium	The negative control response was reported in Fig.1 and appeared adequate. However, the figure was a bit difficult to read.
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is typical of algae acute toxicity tests.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—algal growth in terms of biomass as seen in Fig. 1.
	Metric 18: Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Methods for obtaining biomass were not described in detail other than to say at which time points observations were made.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods and equations for growth were described.
	Metric 22: Reporting of Data	Low	Growth of the marine algae in terms of biomass is presented in Fig. 1. EC10, EC50, EC70, and EC90 values are reported in Fig. 6. Data for each test concentration and control were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on growth of the marine algae *Platymonas subcordiformis*.

Overall Quality Determination

Medium

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.			
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	5257521			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.	
	Metric 5: Negative Control Response	Low	The negative control response was not reported. BCFD values were reported.	
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This appeared adequate to observe BCFD values.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.	
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.	
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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Platymonas subcordiformis</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. BCFD values were reported in Table 1, but little was provided on methods of measuring the test chemical.
Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Calculations for BCFD values were described, but it was not reported how measurements were taken.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods and equations for BCFD were described.
Metric 22:	Reporting of Data	Low	BCFD values were reported in Table 1, but no control responses were reported.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on BCFD of the marine algae *Platymonas subcordiformis*.

Overall Quality Determination

Low

Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.			
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:	Vegetation; Non-vascular Plants; <i>Pyramidomonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5243980			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test chemical was identified by name only.	
	Metric 2: Test Substance Source	Low	The test substance source was not reported, nor was it reported to be analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity and/or grade of test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Low	It was reported that pre-cultures with methanol and without test substances were used as the controls. It was reported that pre-cultures were incubated without chemical until the algal biomass reached 1mg/L, and then the test substances were added. It is unclear if the control value was taken before the addition of chemicals to the other flasks, or if the the pre-cultures without test substance were allowed to continue for the duration of the study.	
	Metric 5: Negative Control Response	High	The biological response of the negative control groups was reported in Figure 1.	
	Metric 6: Randomized Allocation	Low	The study did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The experimental system and/or test media preparation methods were not reported in great detail. The DCB was reported to be prepared by adding 1mL of methanol DCB stock solution to a bottle of double distilled water. Stoppers were used to minimize volatilization. Test concentrations were not measured during the study.	
	Metric 8: Consistency of Exposure Administration	Medium	Test concentrations were not measured, so it is unclear if they remained similar for the duration of the study. Test volumes were not reported. Otherwise, consistency seems adequate.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were justified 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	High	The test organisms were adequately described and were obtained from a reliable source.	

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Study Citation:	Ma, Y. J., Wang, X. L., Yu, W. J., Zhang, L. J., Sun, H. Z. (1997). Toxicity of chlorinated benzenes to marine algae. Chinese Journal of Oceanology and Limnology 15(4):308-313.
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:	Vegetation; Non-vascular Plants; <i>Pyramidomonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5243980

Domain	Metric	Rating	Comments
	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. Pre-culture biomass reached about 1mg/L before test substances were added to initiate the tests.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	Minor uncertainties regarding salinity were identified regarding organism housing, environmental conditions.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22: Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability.
Additional Comments: In this evaluation, growth and growth inhibition were assessed in marine algae species <i>Pyramidomonas sp.</i>			

Overall Quality Determination

Medium

Study Citation:	Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. <i>Chemosphere</i> 35(8):1781-1797.			
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:	Vegetation; Non-vascular Plants; <i>Pyramidomonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	1982535			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The DCB was not certified or analytically verified.	
	Metric 3: Test Substance Purity	Low	The purity was not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors reported that there was a control for the preculture portion of the study for TeCB only. Nothing was reported as a control for the other test chemicals, including o-DCB.	
	Metric 5: Negative Control Response	Uninformative	The negative control response was not reported as it was unclear if there was a negative control for chemicals other than TeCB.	
	Metric 6: Randomized Allocation	Low	The allocation method was not reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	There was a static system with glass stoppers to minimize volatilization. However, exposure concentrations were unclear, and it did not appear that test concentrations were measured at any point.	
	Metric 8: Consistency of Exposure Administration	Low	Exposure concentrations did not appear to be measured, so it is unclear if test concentrations remained consistent for the entire exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Test solutions were made. Analytical measurements were not reported. This was a BCF study.	
	Metric 10: Exposure Duration and Frequency	High	The duration and frequency were appropriate for the test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	A range of test concentrations was given, but the spacing was not reported.	
	Metric 12: Testing at or Below Solubility Limit	High	The range of test concentrations was under the solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The source of the algal species was reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Pre-treatment environmental conditions were well described and equal across groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The biomass of algae was appropriate for algae study, but the number of replicates was not reported.	

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Study Citation: Wang, X. L., Ma, Y. J., Yu, W. J., Geyer, H. J. (1997). Two-compartment thermodynamic model for bioconcentration of hydrophobic organic chemicals by alga: Quantitative relationship between bioconcentration factor and surface area of marine algae or octanol/water partition coefficient. *Chemosphere* 35(8):1781-1797.

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

Health Outcome: ADME (biotransformation)

Chemical: o-Dichlorobenzene

HERO ID: 1982535

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Test conditions were adequate.
	Metric 17: Outcome Assessment Methodology	Uninformative	The acute toxicity test used to validate the BCF model. While BCF was reported, determination of toxicity was not.
	Metric 18: Consistency of Outcome Assessment	High	Outcome assessment was consistent across groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	Variables were consistent for all groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Uninformative	Results were used to fit the BCF model. Statistics or results for acute toxicity were not reported.
	Metric 22: Reporting of Data	Uninformative	Reported data were not sufficient to determine a hazard endpoint.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation is for the BCF of *Pyramidomonas sp.* to o-DCB.

Overall Quality Determination

Uninformative

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiuorong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.			
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:	Vegetation; Non-vascular Plants; <i>Pyramimonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	5257521			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.	
	Metric 5: Negative Control Response	Medium	The negative control response was reported in Fig.1 and appeared adequate. However, the figure was a bit difficult to read.	
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is typical of algae acute toxicity tests.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.	
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.	

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Pyramimonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—algal growth in terms of biomass as seen in Fig. 1.
	Metric 18: Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Methods for obtaining biomass were not described in detail other than to say at which time points observations were made.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical methods and equations for growth were described.
	Metric 22: Reporting of Data	Low	Growth of the marine algae in terms of biomass is presented in Fig. 1. EC10, EC50, EC70, and EC90 values are reported in Fig. 6. Data for each test concentration and control were not reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on growth of the marine algae *Pyramimonas sp.*

Overall Quality Determination

Medium

Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Pyramimonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The o-DCB was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.
	Metric 3: Test Substance Purity	Low	The purity and grade of the test substance were not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of an appropriate negative control that contained <0.01% methanol.
	Metric 5: Negative Control Response	Low	The negative control response was not reported. BCFD values were reported.
	Metric 6: Randomized Allocation	Low	It was not reported how the algae were allocated into study groups.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Test concentrations were prepared by adding 1mL of a methanol stock solution made prior to the experiments to pre-cultures when they reached a biomass of about 1mg/L. Tests were conducted in 250mL flasks. The test volume was not reported. Flasks were closed with glass stoppers to limit volatilization of the test substance, though measurements of the test concentrations were not reported.
	Metric 8: Consistency of Exposure Administration	Medium	The test volume was not reported, but the exposures appeared to be carried out consistently for the duration of the study.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations did not appear to be measured or reported.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This appeared adequate to observe BCFD values.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Exposure concentrations were reported in Table 1 for each test species. There were four test concentrations, and they appeared to be spaced adequately for a response.
	Metric 12: Testing at or Below Solubility Limit	High	Test concentrations were below the water solubility limit.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The source of the test organisms was not reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated prior to the start of the study. Little information was provided on the culturing techniques.
	Metric 15: Number of Organisms and Replicates per Group	Low	It was reported that tests commenced when the biomass of the precultures reached approximately 1mg/L. However, the number of replicates per test concentration was not reported.

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Study Citation:	Xiulin, W., Baotian, S., Ming, G. E., Lei, Z., Chenjian, Z., Xiurong, H. (2002). A model describing the time course and variability in toxicity of hydrophobic chemicals to aquatic organisms. <i>Journal of Ocean University of Qingdao</i> 1(1):71-79.
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:	Vegetation; Non-vascular Plants; <i>Pyramimonas sp.</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	5257521

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Medium	For the tests, algae cells were cultured in f/2 nutrient medium at 20C with 12:12 photoperiod. The culturing conditions of the stock cultures was not reported.
Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology was not clearly reported. BCFD values were reported in Table 1, but little was provided on methods of measuring the test chemical.
Metric 18:	Consistency of Outcome Assessment	Low	Little detail was provided on the execution of the study protocol. Calculations for BCFD values were described, but it was not reported how measurements were taken.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods and equations for BCFD were described.
Metric 22:	Reporting of Data	Low	BCFD values were reported in Table 1, but no control responses were reported.
Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This evaluation was for the effect of o-DCB on BCFD of the marine algae *Pyramimonas sp.*

Overall Quality Determination

Low

Study Citation:	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). Bulletin of Environmental Contamination and Toxicology 54(1):36-42.			
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)			
Taxa, Species, Age:	Vertebrate; Fish; <i>Platichthys flesus</i> ; L.; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	5253054			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test 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	AnalaR grade was used with purity between 97-99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative and solvent controls were used in this study.	
	Metric 5: Negative Control Response	Low	No control results were reported, just LC50 values with 95% confidence intervals.	
	Metric 6: Randomized Allocation	Low	No indication of randomized allocation was reported.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	This was a semi-static test with renewals every 48h. The test substance preparation was reported. Distilled acetone was used as a solvent, and dissolution was further optimized via sonication. Test concentrations were reported to be within 80% of the nominal values.	
	Metric 8: Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentration measurements were not reported, though it was reported that test concentrations were measured and remained within 80% of the nominal.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure was reported and was appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	It was reported there was five experimental exposure groups, but the test concentrations were not reported, so spacing was unclear.	
	Metric 12: Testing at or Below Solubility Limit	Low	Reporting omissions prevented determination of whether exposure concentrations exceeded the water solubility limit.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	There are reservations about the source of test organisms. The test organisms were wild caught, so the health of the organisms was uncertain. The age was not reported, though test organisms of similar sized were used in the study.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	The test organisms were acclimatized to test conditions, and all pretreatment conditions were the same for control and exposed organisms.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The numbers of test organisms and replicates were lower than the typical number for an acute test.	

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Study Citation:	Furay, V. J., Smith, S. (1995). Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (<i>Platichthys flesus</i> L.) and sole (<i>Solea solea</i> L.). <i>Bulletin of Environmental Contamination and Toxicology</i> 54(1):36-42.
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)
Taxa, Species, Age:	Vertebrate; Fish; <i>Platichthys flesus</i> ; L.; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5253054

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Reporting of environmental conditions was not sufficiently reported to evaluate if they were adequate.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Study authors reported that LC50 values were calculated using probit analysis.
Metric 22:	Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment and control group.
Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation was for the LC50 values estimated for *Platichthys flesus* after exposure to o-DCB. This study received an unacceptable rating due to lack of information regarding the test concentrations used and the spacing of the concentrations.

Overall Quality Determination

Uninformative

Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Acartia bifilosa</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
Metric 2:	Test Substance Source	Low	The source of the test substance was not reported.
Metric 3:	Test Substance Purity	Low	No purity was reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control.
Metric 5:	Negative Control Response	Uninformative	Data reported on the control response in Fig. 2 indicates a percent survival ranging from 50-75%, which is not acceptable.
Metric 6:	Randomized Allocation	Low	Allocation was not reported as randomized.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	Solutions were prepared to saturation then diluted to 5 concentrations as percent of saturation (91mg/L). Renewal was not performed. The Petri dishes used were covered with glass cover dishes but were not sealed. Test substances were reported to be measured, but measurements were not reported. Authors report that the 1,2-DCB may have been overestimated in solution because sea water was used, and the solubility was determined for freshwater.
Metric 8:	Consistency of Exposure Administration	Medium	Exposures appeared to be administered consistently across study groups, but details of administration were limited. Test volume was not reported. Test concentration measurements were not reported.
Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were reported to be monitored by spectrophotometer, but they were not reported. EC and LC values are reported as % of saturation. This paper uses 91 mg/L as the saturation value.
Metric 10:	Exposure Duration and Frequency	High	A 4-day observation period was used for this egg hatch and viability study. This appeared adequate for the outcome of interest.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Five concentrations were reported as a percentage of saturation. A control was also reported. Spacing appeared adequate.
Metric 12:	Testing at or Below Solubility Limit	High	Authors used 91 mg/L as the concentration for saturation as previously reported in the literature. Concentrations tested were 10, 5, 1, 0.5, and 0.1% of that value. (Miller, M.M., Ghodbane, S., Wasik, S.P., Tewari, Y.B., Martire, D.E., 1984. Aqueous solubilities, octanol /water partition coefficients, and entropies of melting of chlorinated benzenes and biphenyls. <i>J. Chem. Eng.Data</i> 29, 184-190.) https://pubs.acs.org/doi/10.1021/je00036a027

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Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.		
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)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Acartia bifilosa</i> ; Embryo		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	5246949		
Domain	Metric	Rating	Comments
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	Adults were collected from the wild and eggs from those collections were used. Sampling locations, time of year, and water temperatures were reported. Since the organisms were wild caught, this creates concern regarding organism health.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Conditions prior to the bioassay do not appear to be different between treatments and control.
	Metric 15: Number of Organisms and Replicates per Group	Low	The study reported two replicates for each treatment and control, which is lower than is typical.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Little information was reported on environmental conditions other than a test temperature of 15C at full light and 5C in the dark. Salinity was not reported. Control conditions resulted in 50-75% hatch and viability, which indicates that there are conditions present that affect normal hatch outside of test compound concentrations. No standard method tests were cited for this study.
	Metric 17: Outcome Assessment Methodology	High	Measurements of viability and hatch were adequately described.
	Metric 18: Consistency of Outcome Assessment	High	Assessment was consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was nothing to suggest differences among groups in animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Probit analysis on hatch and viability with means was reported. Percent viability and hatch from each replicate are reported in the figures.
	Metric 22: Reporting of Data	High	Results for this experiment are reported in Figure 2 and Table 4.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported and no limitations within this bioassay were reported.
Additional Comments:	The control mortality for this species in the study indicates that their culture conditions are not appropriate for embryo incubation with this species of copepod.		

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Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Acartia bifilosa</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
Overall Quality Determination		Uninformative	

Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.		
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)		
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Acartia clausi</i> ; Embryo		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	5246949		

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	No purity was reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	Uninformative	Data reported on the control response in Fig. 2 indicates a percent survival and percent viability that are extremely variable (from 0-100%). Study authors reported six control replicates to these issues.
	Metric 6: Randomized Allocation	Low	Allocation was not reported as randomized.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	Solutions were prepared to saturation then diluted to 5 concentrations as percent of saturation (91mg/L). Renewal was not performed. The Petri dishes used were covered with glass cover dishes but were not sealed. Test substances were reported to be measured, but measurements were not reported. Authors report that the 1,2-DCB may have been overestimated in solution because sea water was used, and the solubility was determined for freshwater.
	Metric 8: Consistency of Exposure Administration	Medium	Exposures appeared to be administered consistently across study groups, but details of administration were limited. Test volume was not reported. Test concentration measurements were not reported.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were reported to be monitored by spectrophotometer, but they were not reported. EC and LC values are reported as % of saturation. This paper uses 91 mg/L as the saturation value.
	Metric 10: Exposure Duration and Frequency	High	A 4-day observation period was used for this egg hatch and viability study. This appeared adequate for the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Five concentrations were reported as a percentage of saturation. A control was also reported. Spacing appeared adequate.

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Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Acartia clausi</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
	Metric 12: Testing at or Below Solubility Limit	High	Authors used 91 mg/L as the concentration for saturation as previously reported in the literature. Concentrations tested were 10, 5, 1, 0.5, and 0.1% of that value. (Miller, M.M., Ghodbane, S., Wasik, S.P., Tewari, Y.B., Martire, D.E., 1984. Aqueous solubilities, octanol/water partition coefficients, and entropies of melting of chlorinated benzenes and biphenyls. <i>J. Chem. Eng. Data</i> 29, 184-190.) https://pubs.acs.org/doi/10.1021/je00036a027
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	Adults were collected from the wild and eggs from those collections were used. Sampling locations, time of year, and water temperatures were reported. Since the organisms were wild caught, this creates concern regarding organism health.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Conditions prior to the bioassay do not appear to be different between treatments and control.
	Metric 15: Number of Organisms and Replicates per Group	Low	The study reports 3-6 replicates were used for each treatment and control.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Little information was reported on environmental conditions other than a test temperature of 15C at full light and 5C in the dark. Salinity was not reported. Control conditions resulted in 0-100% hatch and viability, which indicates that there are conditions present that affect normal hatch outside of test compound concentrations. No standard method tests were cited for this study.
	Metric 17: Outcome Assessment Methodology	High	Measurements of viability and hatch were adequately described.
	Metric 18: Consistency of Outcome Assessment	High	Assessment was consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was nothing to suggest differences among groups in animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Probit analysis on hatch and viability with means was reported. Percent viability and hatch from each replicate are reported in the figures.
	Metric 22: Reporting of Data	High	Results for this experiment are reported in Figure 2 and Table 4.

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Study Citation: Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. *Journal of Experimental Marine Biology and Ecology* 242(1):59-74.
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)
Taxa, Species, Age: Invertebrate; Arthropods; *Acartia clausi*; Embryo
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 5246949

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported and no limitations within this bioassay were reported.

Additional Comments: The control mortality for this species in the study indicates that their culture conditions are not appropriate for embryo incubation with this species of copepod. % hatch and % viability ranged from 0% to 100% (Figure 2). The study had 6 control replicates due to these embryo survival problems.

Overall Quality Determination

Uninformative

Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.
	Metric 3: Test Substance Purity	Low	No purity was reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.
	Metric 5: Negative Control Response	High	All control groups had high survival (>90%). Results are reported in Table 1.
	Metric 6: Randomized Allocation	Low	Allocation was not reported as randomized.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	Solutions were prepared to saturation. Renewal was not performed. The Petri dishes used were covered with glass cover dishes but were not sealed. Test substances were reported to be measured, but measurements were not reported. Authors report that the 1,2-DCB may have been overestimated in solution because sea water was used, and the solubility was determined for freshwater.
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration was inconsistent for the saturation study. Some groups were incubated "overnight" with no hours of exposure reported, while others remained in the test solution throughout the bioassay.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were reported to be monitored by spectrophotometer, but they were not reported. Nominal concentrations were reported.
	Metric 10: Exposure Duration and Frequency	High	A 4-day observation period was used for this egg hatch and viability study. This appeared adequate for the outcome of interest.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This experiment was only conducted on a single concentration of the test compound that the authors reported as saturation administered in a short duration ("overnight") or throughout embryo incubation.
	Metric 12: Testing at or Below Solubility Limit	High	Authors used 91 mg/L as the concentration for saturation as previously reported in the literature (Miller, M.M., Ghodbane, S., Wasik, S.P., Tewari, Y.B., Martire, D.E., 1984. Aqueous solubilities, octanol /water partition coefficients, and entropies of melting of chlorinated benzenes and biphenyls. <i>J. Chem. Eng.Data</i> 29, 184–190.) https://pubs.acs.org/doi/10.1021/je00036a027
Domain 4: Test Organism			

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Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
	Metric 13: Test Organism Characteristics	Low	Adults were collected from the wild and eggs from those collections were used. Sampling locations, time of year, and water temperatures were reported. Since the organisms were wild caught, this creates concern regarding organism health.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Conditions prior to the bioassay do not appear to be different between treatments and control.
	Metric 15: Number of Organisms and Replicates per Group	Low	The study reports 3-5 replicates were used for each treatment and control. The number of eggs per replicate was not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Little information was reported on environmental conditions other than a test temperature of 15C at full light and 5C in the dark. Salinity was not reported.
	Metric 17: Outcome Assessment Methodology	High	Measurements of viability and hatch were adequately described.
	Metric 18: Consistency of Outcome Assessment	High	Assessment was consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was nothing to suggest differences among groups in animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	This experiment only calculated percentage of hatch and viability with means and standard deviations from arcsine-transformed data.
	Metric 22: Reporting of Data	High	Results for this experiment are reported in Table 1.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported and no limitations within this bioassay were reported.
Additional Comments:	This study within the publication was conducted on a single concentration of the test compound that the authors reported as saturation administered in a short duration ("overnight") or throughout embryo incubation. The saturation concentration used was 91 mg/L.		

Overall Quality Determination**Medium**

Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.			
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)			
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Embryo			
Health Outcome:	Mortality			
Chemical:	o-Dichlorobenzene			
HERO ID:	5246949			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified by name only.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported.	
	Metric 3: Test Substance Purity	Low	No purity was reported.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a concurrent negative control.	
	Metric 5: Negative Control Response	High	All control groups had high survival (>90%).	
	Metric 6: Randomized Allocation	Low	Allocation was not reported as randomized.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Solutions were prepared to saturation then diluted to 5 concentrations as percent of saturation (91mg/L). Renewal was not performed. The Petri dishes used were covered with glass cover dishes but were not sealed. Test substances were reported to be measured, but measurements were not reported. Authors report that the 1,2-DCB may have been overestimated in solution because sea water was used, and the solubility was determined for freshwater.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposures appeared to be administered consistently across study groups, but details of administration were limited. Test volume was not reported. Test concentration measurements were not reported.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were reported to be monitored by spectrophotometer, but they were not reported. EC and LC values are reported as % of saturation. This paper uses 91 mg/L as the saturation value.	
	Metric 10: Exposure Duration and Frequency	High	A 4-day observation period was used for this egg hatch and viability study. This appeared adequate for the outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	Five concentrations were reported as a percentage of saturation. A control was also reported. Spacing appeared adequate.	
	Metric 12: Testing at or Below Solubility Limit	High	Authors used 91 mg/L as the concentration for saturation as previously reported in the literature. Concentrations tested were 10, 5, 1, 0.5, and 0.1% of that value. (Miller, M.M., Ghodbane, S., Wasik, S.P., Tewari, Y.B., Martire, D.E., 1984. Aqueous solubilities, octanol /water partition coefficients, and entropies of melting of chlorinated benzenes and biphenyls. <i>J. Chem. Eng.Data</i> 29, 184–190.) https://pubs.acs.org/doi/10.1021/je00036a027	
Domain 4: Test Organism				

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Study Citation:	Lindley, J. A., Donkin, P., Evans, S. V., George, C. L., Uil, K. F. (1999). Effects of two organochlorine compounds on hatching and viability of calanoid copepod eggs. <i>Journal of Experimental Marine Biology and Ecology</i> 242(1):59-74.
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)
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Eurytemora affinis</i> ; Embryo
Health Outcome:	Mortality
Chemical:	o-Dichlorobenzene
HERO ID:	5246949

Domain	Metric	Rating	Comments
	Metric 13: Test Organism Characteristics	Low	Adults were collected from the wild and eggs from those collections were used. Sampling locations, time of year, and water temperatures were reported. Since the organisms were wild caught, this creates concern regarding organism health.
	Metric 14: Acclimatization and Pretreatment Conditions	High	Conditions prior to the bioassay do not appear to be different between treatments and control.
	Metric 15: Number of Organisms and Replicates per Group	Low	The study reported two replicates for each treatment and control, which is lower than is typical.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Little information was reported on environmental conditions other than a test temperature of 15C at full light and 5C in the dark. Salinity was not reported.
	Metric 17: Outcome Assessment Methodology	High	Measurements of viability and hatch were adequately described.
	Metric 18: Consistency of Outcome Assessment	High	Assessment was consistent among treatment and control groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Little was reported on environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was nothing to suggest differences among groups in animal attrition or health outcomes.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Probit analysis on hatch and viability with means was reported. Percent viability and hatch from each replicate are reported in the figures.
	Metric 22: Reporting of Data	High	Results for this experiment are reported in Figure 2 and Table 4.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported and no limitations within this bioassay were reported.
Additional Comments: The dose-response data for this study represent % dilutions from saturation, which is reported as 91 mg/L for this study. The study was completed with two replicates per treatment concentration and there was no renewal of the test compound throughout time.			

Overall Quality Determination**Medium**

Study Citation:	Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm <i>Eisenia fetida</i> . <i>Journal of Environmental Quality</i> 14(3):383-388.		
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); <i>Eisenia fetida</i> ; Savigny; Adult		
Health Outcome:	Mortality		
Chemical:	o-Dichlorobenzene		
HERO ID:	3625226; Linked HERO ID(s): 3625226, 5094945		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	High	The test substance was identified by CASRN.
Metric 2:	Test Substance Source	Low	Three sources were reported for the chemicals tested in this study. It was not specified which test chemical came from which source. The test chemical was not reported to be analytically verified by the performing laboratory.
Metric 3:	Test Substance Purity	High	All test chemicals were reported to have a purity of 98% or greater.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Study authors reported the use of an appropriate concurrent negative control.
Metric 5:	Negative Control Response	Low	The negative control response was not reported. Only LC50 values were reported.
Metric 6:	Randomized Allocation	Low	It was not reported how organisms were allocated into study groups.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test concentrations was not described in great detail. The test system consisted of glass vials that were 8cm long and 3cm in diameter with one worm per vial. Chemicals were applied to the filter paper using a solvent, either chloroform or acetone, but it was not specified which was used for each chemical.
Metric 8:	Consistency of Exposure Administration	High	It appears that the exposures were administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	It was determined gravimetrically that organic chemicals added to the vials with organic solvents didn't volatilize with organic solvent. However, loss of test substance did not appear to be analyzed for the study duration.
Metric 10:	Exposure Duration and Frequency	High	The study duration was reported to be 48h. This is an appropriate duration for an earthworm filter paper test according to OECD guidelines.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	It was reported that there were at least five test concentrations evaluated for each test substance. However, the actual concentrations used were not reported, so spacing could not be determined.
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via filter paper.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	The earthworms were from in-house cultures. Adults were used for this test.
Metric 14:	Acclimatization and Pretreatment Conditions	High	Culture conditions and test conditions appeared to be similar enough that an acclimation period would not be necessary.

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Study Citation: Neuhauser, E. F., Loehr, R. C., Malecki, M. R., Milligan, D. L., Durkin, P. R. (1985). The toxicity of selected organic chemicals to the earthworm *Eisenia fetida*. *Journal of Environmental Quality* 14(3):383-388.
Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path: Terrestrial; N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Savigny; Adult
Health Outcome: Mortality
Chemical: o-Dichlorobenzene
HERO ID: 3625226; Linked HERO ID(s): 3625226, 5094945

Domain	Metric	Rating	Comments
	Metric 15: Number of Organisms and Replicates per Group	Medium	It was reported that there was one worm per vial with at least 10 replicates per test concentration. This is in accordance with OECD guidelines.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Worms were kept in vials with one worm per vial at a temperature of 20C in the dark for the duration of the study.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest—mortality to estimate an LC50 value.
	Metric 18: Consistency of Outcome Assessment	High	The outcomes were assessed consistently across study groups. All earthworms were considered dead if they did not respond to gentle mechanical touch.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	LC50 values were determined using the method of Litchfield and Wilcoxon.
	Metric 22: Reporting of Data	Low	Only LC50 values were reported. Data for each concentration was not reported, nor was control data.
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Confidence intervals were reported in Table 1.
Additional Comments:	This evaluation was for the effect of o-DCB on the earthworm <i>E. fetida</i> . This was an acute test that used filter paper for the exposure. LC50 values were estimated.		

Overall Quality Determination

High

Study Citation:	Kraaij, H., Connell, D. W. (1997). Bioconcentrations and uptake kinetics of chlorobenzenes in soy-bean roots. <i>Chemosphere</i> 34(12):2607-2620.			
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
Exposure Route, Media, Path:	Terrestrial; Water, Cell Culture Media; Root uptake			
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Glycine max</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	ADME (biotransformation)			
Chemical:	o-Dichlorobenzene			
HERO ID:	5265635			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test 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 purity was reported as $\geq 98\%$.	
Domain 2: Test Design				
	Metric 4: Negative Controls	Uninformative	Study authors did not report the use of a negative control. A 0h uptake was reported, but no concurrent negative control was reported.	
	Metric 5: Negative Control Response	Uninformative	A negative control was not reported, so there was not a negative control response.	
	Metric 6: Randomized Allocation	Low	Researchers did not report how organisms were allocated to study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Little detail was provided on the preparation of the test solution. Several solvents were reported, but it was not specified which solvent was used for which test substance.	
	Metric 8: Consistency of Exposure Administration	Low	Reporting omissions are likely to have a substantial impact on results. Test container size and volume of test solutions were not reported. Little was reported on environmental conditions.	
	Metric 9: Measurement of Test Substance Concentration	High	Exposure concentrations were measured and are similar to nominal concentrations ($< 5\%$ difference), though actual measurement values were not reported.	
	Metric 10: Exposure Duration and Frequency	High	The duration of exposure and/or exposure frequency were reported and appropriate for the study type.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Both experiments were time related with only one concentration.	
	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	Low	The source of the soybeans was not reported. It is unclear if they were from in-house or sourced from somewhere else.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the plants were acclimated to test conditions.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	1.5g of roots was placed in each test chamber, and there were three replicates for each experiment.	
Domain 5: Outcome Assessment				

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Study Citation: Kraaij, H., Connell, D. W. (1997). Bioconcentrations and uptake kinetics of chlorobenzenes in soy-bean roots. *Chemosphere* 34(12):2607-2620.
Duration: Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path: Terrestrial; Water, Cell Culture Media; Root uptake
Taxa, Species, Age: Vegetation; Vascular Plants; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: ADME (biotransformation)
Chemical: o-Dichlorobenzene
HERO ID: 5265635

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Little information was provided on the environmental conditions of the soybean roots. Test temperature and lightning conditions were not reported, nor was pH of the culture medium.
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology reported the intended outcome.
	Metric 18: Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	Statistical analysis was not possible. Formulas and methods for determining uptake were reported.
	Metric 22: Reporting of Data	Low	Data for exposure-related findings were not shown for each treatment or time.
	Metric 23: Explanation of Unexpected Outcomes	Low	Study authors did not report measures of variability.
Additional Comments:	This evaluation was for the uptake of o-DCB in soybean roots after hydroponic exposure. Two tests were run, one at 5,000ug/L and one at 10,00ug/L. This study received an unacceptable rating due the lack of reporting of a negative control. A baseline at 0h was reported for another chemicals, but this seems inefficient for a negative control.		

Overall Quality Determination

Uninformative

Study Citation:	Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. <i>Chemosphere</i> 53(5):583-591.		
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days		
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake		
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Holcus lanatus</i> ; Adult		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	1003825		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test chemical was only identified by name.
Metric 2:	Test Substance Source	Low	The test substance was obtained from Sigma. It was not reported that the test chemical was analytically verified.
Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Negative controls were used and results reported.
Metric 5:	Negative Control Response	High	Negative controls were used and results reported.
Metric 6:	Randomized Allocation	Low	Allocation of test organisms was not reported.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was well described. A closed system was used.
Metric 8:	Consistency of Exposure Administration	Medium	Authors mentioned that the lower recovery of o-dichlorobenzene was probably due to its relatively high volatility and possible losses during spiking procedures. So, there could have been some inconsistencies between replicates due to this.
Metric 9:	Measurement of Test Substance Concentration	High	A scintillation counter was used to measure the test concentrations throughout the experiment. The mass balance recovery for total C14 test substance added to the system was an average of 91%.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration and frequency were appropriate for this experiment. Plants were harvested and analyzed at two weeks post initiation.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one concentration of o-dichlorobenzene used.
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Test organisms (grass) were adequately described and obtained from Herbiseed, UK.
Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Minimal details were given. Plants were grown in 100mL Erlenmeyer flasks until a mature root system was developed (6 weeks). Containers were placed in a microcosm apparatus for the test without disturbing the plants.
Metric 15:	Number of Organisms and Replicates per Group	Low	There were two spiked and two unspiked plants for the test with o-dichlorobenzene.

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Study Citation:	Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. Chemosphere 53(5):583-591.
Duration:	Overall Duration: > 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Holcus lanatus</i> ; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1003825

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate for the test.
	Metric 17: Outcome Assessment Methodology	High	The outcome methodology was reported.
	Metric 18: Consistency of Outcome Assessment	High	Assessments were consistent across all groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Minimal details were reported on how the plants were raised prior to the test start. Environmental conditions during the test were reported and adequate.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences in plants between the spiked and unspiked that could have influenced the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	There was only one concentration tested and a control group.
	Metric 22: Reporting of Data	High	Results were presented in Table 1.
	Metric 23: Explanation of Unexpected Outcomes	High	Standard deviations were shown in Table 1.

Additional Comments: This evaluation form is for the assessment of plants after two weeks of exposure.

Overall Quality Determination High

Study Citation:	Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. <i>Chemosphere</i> 53(5):583-591.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake		
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Holcus lanatus</i> ; Adult		
Health Outcome:	ADME (biotransformation)		
Chemical:	o-Dichlorobenzene		
HERO ID:	1003825		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
Metric 1:	Test Substance Identity	Low	The test chemical was only identified by name.
Metric 2:	Test Substance Source	Low	The test substance was obtained from Sigma. It was not reported that the test chemical was analytically verified.
Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design			
Metric 4:	Negative Controls	High	Negative controls were used and results reported.
Metric 5:	Negative Control Response	High	Negative controls were used and results reported.
Metric 6:	Randomized Allocation	Low	Allocation of test organisms was not reported.
Domain 3: Exposure Characterization			
Metric 7:	Experimental System/Test Media Preparation	High	The experimental system was well described. A closed system was used.
Metric 8:	Consistency of Exposure Administration	Medium	Authors mentioned that the lower recovery of o-dichlorobenzene was probably due to its relatively high volatility and possible losses during spiking procedures. So, there could have been some inconsistencies between replicates due to this.
Metric 9:	Measurement of Test Substance Concentration	High	A scintillation counter was used to measure the test concentrations throughout the experiment. The mass balance recovery for total C14 test substance added to the system was an average of 91%.
Metric 10:	Exposure Duration and Frequency	High	The exposure duration and frequency were appropriate for this experiment. Plants were harvested and analyzed at five weeks post initiation.
Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one concentration of o-dichlorobenzene used.
Metric 12:	Testing at or Below Solubility Limit	N/A	The exposure was via soil.
Domain 4: Test Organism			
Metric 13:	Test Organism Characteristics	High	Test organisms (grass) were adequately described and obtained from Herbiseed, UK.
Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Minimal details were given. Plants were grown in 100mL Erlenmeyer flasks until a mature root system was developed (6 weeks). Containers were placed in a microcosm apparatus for the test without disturbing the plants.
Metric 15:	Number of Organisms and Replicates per Group	Low	There were two spiked and two unspiked plants for the test with o-dichlorobenzene.

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Study Citation:	Wilson, S. (2003). Investigation of organic xenobiotic transfers, partitioning and processing in air–soil–plant systems using a microcosm apparatus. Part II: comparing the fate of chlorobenzenes in grass planted soil. <i>Chemosphere</i> 53(5):583-591.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; <i>Holcus lanatus</i> ; Adult
Health Outcome:	ADME (biotransformation)
Chemical:	o-Dichlorobenzene
HERO ID:	1003825

Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	High	Environmental conditions were adequate for the test.
	Metric 17: Outcome Assessment Methodology	High	The outcome methodology was reported.
	Metric 18: Consistency of Outcome Assessment	High	Assessments were consistent across all groups.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	Minimal details were reported on how the plants were raised prior to the test start. Environmental conditions during the test were reported and adequate.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There were no reported differences in plants between the spiked and unspiked that could have influenced the outcome assessment.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	N/A	There was only one concentration tested and a control group.
	Metric 22: Reporting of Data	High	Results were presented in Table 1.
	Metric 23: Explanation of Unexpected Outcomes	High	Standard deviations were shown in Table 1.

Additional Comments: This evaluation form is for the assessment of plants after five weeks of exposure.

Overall Quality Determination High

Study Citation:	Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. <i>Environmental Toxicology and Chemistry</i> 17(8):1462-1468.			
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Other; Fungus; <i>Kingdom Fungi</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	2725058			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified as dichlorobenzene. No CASRN was included.	
	Metric 2: Test Substance Source	Low	The manufacturer was identified as Sigma for the o-dichlorobenzene. It was not reported to be analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity of the chemical was >99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were included in the study.	
	Metric 5: Negative Control Response	Medium	The response of the control was reported with limited details.	
	Metric 6: Randomized Allocation	Low	Fungi were used in mixed populations in the experiments, but allocation was not reported on specifically.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The preparation method of the dosed soil was not reported in detail. The test chambers and test conditions were described adequately.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported as consistent, but exposure concentrations were not measured, so it is unclear if the test concentrations remained steady for the exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was appropriate for the study.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	There were only two exposure groups for this portion of the study.	
	Metric 12: Testing at or Below Solubility Limit	N/A	This exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Uninformative	Individual species were not identified in the population used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report acclimatization or pretreatment.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of individual organisms was not reported. The study was completed in triplicate.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Soil conditions were reported.	

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Study Citation:	Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. <i>Environmental Toxicology and Chemistry</i> 17(8):1462-1468.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Other; Fungus; <i>Kingdom Fungi</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	o-Dichlorobenzene
HERO ID:	2725058

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	Fungal hyphal length was reported, and was one of the outcomes of interest.
	Metric 18: Consistency of Outcome Assessment	High	No reports of inconsistency were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	P-values and standard error of the mean were reported, but methods were not reported.
	Metric 22: Reporting of Data	High	Fungal hyphal length was addressed and reported for the exposure levels and the control in Fig. 1b.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: The paper did not identify any specific fungal species, rather they referred to the population as a whole throughout the study. Methodology for staining of fungi was cited from Soderstrom BE. 1977. Vital staining of fungi in pure cultures and in soil was performed with fluorescein diacetate. *Soil Biol Biochem* 9:59-63.

Overall Quality Determination**Uninformative**

Study Citation:	Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. <i>Environmental Toxicology and Chemistry</i> 17(8):1462-1468.			
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days			
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Other; Fungus; <i>Kingdom Fungi</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Development/Growth			
Chemical:	o-Dichlorobenzene			
HERO ID:	2725058			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	The test substance was identified as dichlorobenzene. No CASRN was included.	
	Metric 2: Test Substance Source	Low	The manufacturer was identified as Sigma for the o-dichlorobenzene. It was not reported to be analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	The purity of the chemical was >99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Negative controls were included in the study.	
	Metric 5: Negative Control Response	Medium	The response of the control was reported with limited details.	
	Metric 6: Randomized Allocation	Low	Fungi were used in mixed populations in the experiments, but allocation was not reported on specifically.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	The preparation method of the dosed soil was not reported in detail. The test chambers and test conditions were described adequately.	
	Metric 8: Consistency of Exposure Administration	Medium	Exposure administration was reported as consistent, but exposure concentrations were not measured, so it is unclear if the test concentrations remained steady for the exposure duration.	
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured or reported.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was appropriate for the study.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A	There was only one exposure concentration for this portion of the study as the goal was not to observe a dose response.	
	Metric 12: Testing at or Below Solubility Limit	N/A	This exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Uninformative	Individual species were not identified in the population used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report acclimatization or pretreatment.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of individual organisms was not reported. The study was completed in triplicate.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	Medium	Soil conditions were reported.	

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Study Citation: Meharg, A. A., Wyatt, C. L., Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. (1998). Response of soil microbial biomass to 1,2-dichlorobenzene addition in the presence of plant residues. *Environmental Toxicology and Chemistry* 17(8):1462-1468.

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

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

Taxa, Species, Age: Other; Fungus; *Kingdom Fungi*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Development/Growth

Chemical: o-Dichlorobenzene

HERO ID: 2725058

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	High	Fungal hyphal length was reported, and was one of the outcomes of interest.
	Metric 18: Consistency of Outcome Assessment	High	No reports of inconsistency were reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	P-values and standard error of the mean were reported, but methods were not reported.
	Metric 22: Reporting of Data	High	Fungal hyphal length was addressed and reported for the exposure levels and the control in Fig. 3b.
	Metric 23: Explanation of Unexpected Outcomes	High	No unexpected outcomes were reported.

Additional Comments: The paper did not identify any specific fungal species, rather they referred to the population as a whole throughout the study. Methodology for staining of fungi was cited from Soderstrom BE. 1977. Vital staining of fungi in pure cultures and in soil was performed with fluorescein diacetate. *Soil Biol Biochem* 9:59-63.

Overall Quality Determination

Uninformative

Study Citation:	Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. <i>Environmental Toxicology and Chemistry</i> 18(9):1891-1898.			
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days			
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa, Species, Age:	Other; Fungus; <i>Name not given</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic-Nutritional and Metabolic			
Chemical:	o-Dichlorobenzene			
HERO ID:	5256918			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	Low	Only the chemical name was given.	
	Metric 2: Test Substance Source	Low	The source was reported but the test substance was not analytically verified.	
	Metric 3: Test Substance Purity	High	Percent purity was reported to be >99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A negative control was used in the experiment.	
	Metric 5: Negative Control Response	High	Biological response of the control was adequate.	
	Metric 6: Randomized Allocation	Low	Random allocation was not specifically mentioned in the paper.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The test for metabolic potential was described in some detail on page 1894 of the pdf.	
	Metric 8: Consistency of Exposure Administration	High	Exposure administration seemed consistent across treatment groups.	
	Metric 9: Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured at the beginning and end of the experiment in the test flasks. However, the concentration of the test substance in various soil fractions in another set of identical microcosms was determined at the end of the experiment.	
	Metric 10: Exposure Duration and Frequency	High	Samples were incubated for a total of eight days for the metabolic potential test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups was adequate.	
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	Test organisms (fungus in soil) were not described in detail.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were similar for control and exposed groups.	
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of fungi in the soil were not reported. Three control replicates were used, and it sounds like there may have been two replicates per treatment group but that wasn't entirely clear.	
Domain 5: Outcome Assessment				
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Study Citation: Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. *Environmental Toxicology and Chemistry* 18(9):1891-1898.

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

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

Taxa, Species, Age: Other; Fungus; *Name not given*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Mechanistic-Nutritional and Metabolic

Chemical: o-Dichlorobenzene

HERO ID: 5256918

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not reported.
	Metric 17: Outcome Assessment Methodology	High	On each occasion plates were scored on a four-point scale(0, 1, 2, and 3) relative to the extent of coloration in each welland therefore cellular metabolism.
	Metric 18: Consistency of Outcome Assessment	Medium	Some details were limited so consistency of outcome assessment may be inadequate.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported, and no pretreatment conditions were given either.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to the exposure were reported in the paper.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was described well.
	Metric 22: Reporting of Data	Medium	Figure 4 shows the metabolic potential results. No raw data was shown.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes. Measures of variability were given in Figure 4.

Additional Comments: None

Overall Quality Determination

Medium

Study Citation:	Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. <i>Environmental Toxicology and Chemistry</i> 18(9):1891-1898.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Other; Fungus; <i>Name not given</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5256918		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Only the chemical name was given.
	Metric 2: Test Substance Source	Low	The source was reported but the test substance was not analytically verified.
	Metric 3: Test Substance Purity	High	Percent purity was reported to be >99%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A negative control was used in the experiment.
	Metric 5: Negative Control Response	High	Biological response of the control was adequate.
	Metric 6: Randomized Allocation	Low	Random allocation was not specifically mentioned in the paper.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	Experiments were done in 500ml flask with lids. Dry spiking of soils was conducted. Fate of the test substance in another set of identically prepared microcosms was determined at the end of the experiment.
	Metric 8: Consistency of Exposure Administration	High	Exposure administration was consistent across treatment groups.
	Metric 9: Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured at the beginning and end of the experiment in the test flasks. However, the concentration of the test substance in various soil fractions in another set of identical microcosms was determined at the end of the experiment.
	Metric 10: Exposure Duration and Frequency	High	It was a microcosm study that lasted a total 49 days.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups was adequate.
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	Test organisms (fungus in soil) were not described in detail.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions were similar for control and exposed groups.
	Metric 15: Number of Organisms and Replicates per Group	Low	The number of fungi in the soil were not reported. Three microcosms were used per treatment.
Domain 5: Outcome Assessment			
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Study Citation: Thompson, I. P., Bailey, M. J., Boyd, E. M., Maguire, N., Meharg, A. A., Ellis, R. J. (1999). Concentration effects of 1,2-dichlorobenzene on soil microbiology. *Environmental Toxicology and Chemistry* 18(9):1891-1898.

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

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

Taxa, Species, Age: Other; Fungus; *Name not given*; Not Applicable (e.g., fungi or algae studies) or Not Reported

Health Outcome: Development/Growth

Chemical: o-Dichlorobenzene

HERO ID: 5256918

Domain	Metric	Rating	Comments
	Metric 16: Adequacy of Test Conditions	Low	Environmental conditions were not reported.
	Metric 17: Outcome Assessment Methodology	High	Fungal hyphal length was determined by vital staining with fluorescein diacetate.
	Metric 18: Consistency of Outcome Assessment	Low	There was not enough details given to determine whether outcome assessment was consistent across study groups and controls.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported, and no pretreatment conditions were given either.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to the exposure were reported in the paper.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	High	Statistical analysis was described well.
	Metric 22: Reporting of Data	Medium	Data (Fungal hyphae length; via a figure) were shown for each treatment group and control. No raw data was reported.
	Metric 23: Explanation of Unexpected Outcomes	High	There were no unexpected outcomes. Measures of variability were given in Figure 2.
Additional Comments: The fungal growth in soil exposed to o-dichlorobenzene was reported using a figure with data for control group and measures of variability clearly depicted. The species/type of fungi, environmental conditions during the test, and the number and fungal hyphae length at the beginning of the experiment were not reported.			

Overall Quality Determination

Low

Study Citation:	Liang, H., Chen, A., Ding, C., Li, Z. (2011). Influence of 1,2-Dichlorobenzene on Microbial and Enzyme Activities in Wetland Soil. <i>Procedia Environmental Sciences</i> 10:122-127.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Other; Fungus; <i>No name given</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Development/Growth		
Chemical:	o-Dichlorobenzene		
HERO ID:	5262745		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Only the chemical name was given.
	Metric 2: Test Substance Source	Low	The test chemical source was not reported, and test substance identity was not analytically verified.
	Metric 3: Test Substance Purity	Low	Percent purity of the test substance was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A negative control was used in the experiment.
	Metric 5: Negative Control Response	Medium	The total number of fungi in the negative control on Day 0 was not counted. The negative control data was presented from days 7 through 50.
	Metric 6: Randomized Allocation	Low	Random allocation was not mentioned in the study.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study does not provide details on test media preparation. The concentration of test substance in the soils was not measured at any point throughout the study.
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration was not reported in detail so there is uncertainty surrounding consistency.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration seemed appropriate to meet study objectives.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four treatment groups and one control group used. A significant effect was observed with the treatment concentrations used.
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The species/type of fungi was not reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Pretreatment conditions were not adequately reported. Moisture content of the soil was not measured.
	Metric 15: Number of Organisms and Replicates per Group	Low	Replicates were not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Soil characteristics- pH, organic matter content, moisture content, etc. were not reported in the control and treatment groups.

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Study Citation: Liang, H., Chen, A., Ding, C., Li, Z. (2011). Influence of 1,2-Dichlorobenzene on Microbial and Enzyme Activities in Wetland Soil. *Procedia Environmental Sciences* 10:122-127.
Duration: Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path: Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Other; Fungus; *No name given*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Development/Growth
Chemical: o-Dichlorobenzene
HERO ID: 5262745

Domain	Metric	Rating	Comments
	Metric 17: Outcome Assessment Methodology	Low	Outcome assessment methodology was not described well.
	Metric 18: Consistency of Outcome Assessment	Low	The study protocol for outcome assessment was not adequately reported.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Soil characteristics- pH, organic matter content, moisture content, all of which could affect exposure concentrations were not reported for the control and treatment groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to the exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	The authors used a t-test, but since the experiment has multiple groups, they should have used ANOVA.
	Metric 22: Reporting of Data	Medium	Table 2 showed the number of fungi for each concentration. It is assumed the value is an average but that is not explicitly stated. No raw data was reported.
	Metric 23: Explanation of Unexpected Outcomes	Low	Measures of variability were not shown in Table 2.

Additional Comments: Details on test media preparation and exposure administration were limited. Exposure concentrations were not measured, and replicates were not reported.

Overall Quality Determination **Low**

Study Citation:	Liang, H., Chen, A., Ding, C., Li, Z. (2011). Influence of 1,2-Dichlorobenzene on Microbial and Enzyme Activities in Wetland Soil. <i>Procedia Environmental Sciences</i> 10:122-127.		
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days		
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
Taxa, Species, Age:	Other; Fungus; <i>No name given</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported		
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)		
Chemical:	o-Dichlorobenzene		
HERO ID:	5262745		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	Low	Only the chemical name was given.
	Metric 2: Test Substance Source	Low	The test chemical source was not reported, and test substance identity was not analytically verified.
	Metric 3: Test Substance Purity	Low	Percent purity of the test substance was not reported.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A negative control was used in the experiment.
	Metric 5: Negative Control Response	Medium	The text for Figures 1 and 2 describes the control responses in relation to the treatments, but it is unclear which bars in the figures are representing the control data (not labeled).
	Metric 6: Randomized Allocation	Low	Random allocation was not mentioned in the study.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Low	The study does not provide details on test media preparation. The concentration of test substance in the soils was not measured at any point throughout the study.
	Metric 8: Consistency of Exposure Administration	Low	Exposure administration was not reported in detail so there is uncertainty surrounding consistency.
	Metric 9: Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10: Exposure Duration and Frequency	High	The exposure duration seemed appropriate to meet study objectives.
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four treatment groups and one control group used. A significant effect was observed with the treatment concentrations used.
	Metric 12: Testing at or Below Solubility Limit	N/A	Exposure was via soil.
Domain 4: Test Organism			
	Metric 13: Test Organism Characteristics	Low	The species/type of fungi was not reported.
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Pretreatment conditions were not adequately reported. Moisture content of the soil was not measured.
	Metric 15: Number of Organisms and Replicates per Group	Low	Replicates were not reported.
Domain 5: Outcome Assessment			
	Metric 16: Adequacy of Test Conditions	Low	Soil characteristics- pH, organic matter content, moisture content, etc. were not reported in the control and treatment groups.
	Metric 17: Outcome Assessment Methodology	Medium	There was some information provided on the methods for assessing urease and catalase activity.

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Study Citation: Liang, H., Chen, A., Ding, C., Li, Z. (2011). Influence of 1,2-Dichlorobenzene on Microbial and Enzyme Activities in Wetland Soil. *Procedia Environmental Sciences* 10:122-127.
Duration: Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path: Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Other; Fungus; *No name given*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical: o-Dichlorobenzene
HERO ID: 5262745

Domain	Metric	Rating	Comments
	Metric 18: Consistency of Outcome Assessment	Medium	The study protocol for outcome assessment lacked some details.
Domain 6: Confounding / Variable Control			
	Metric 19: Confounding Variables in Test Design and Procedures	Low	Soil characteristics- pH, organic matter content, moisture content, all of which could affect exposure concentrations were not reported for the control and treatment groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	No outcomes unrelated to the exposure were reported.
Domain 7: Data Presentation and Analysis			
	Metric 21: Statistical Methods	Low	The authors used a t-test, but since the experiment has multiple groups, they should have used ANOVA.
	Metric 22: Reporting of Data	Medium	Urease activity was shown in Figure 1 and catalase activity was shown in Figure 2. No raw data was provided.
	Metric 23: Explanation of Unexpected Outcomes	Low	Measures of variability were not shown in Figure 1 or 2.

Additional Comments: Details on test media preparation and exposure administration were limited. Exposure concentrations were not measured, and replicates were not reported.

Overall Quality Determination **Low**