



United States  
Environmental Protection Agency

Office of Chemical Safety and  
Pollution Prevention

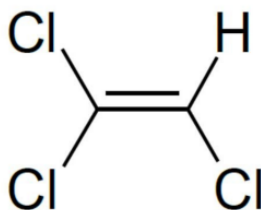
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# Final Risk Evaluation for Trichloroethylene

## Systematic Review Supplemental File:

### Data Quality Evaluation of Environmental Hazard Studies

CASRN: 79-01-6



*November 2020*

# Table of Contents

<b>HERO ID</b>	Data Type	Reference	<b>1</b>
<b>7508</b>	Acute (0-96 hour); Aquatic; Invertebrates	Leblanc, G. A.. 1980. Acute toxicity of priority pollutants to water flea ( <i>Daphnia magna</i> ). <i>Bulletin of Environmental Contamination and Toxicology</i> 24:684-691	<b>1</b>
<b>18050</b>	Other; Aquatic; Fish	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> ).	<b>5</b>
<b>18064</b>	Acute (0-96 hour); Aquatic; Fish	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> 26:446-452	<b>9</b>
<b>32169</b>	Acute (0-96 hour); Aquatic; Fish	Geiger, D. L.,Northcott, C. E.,Call, D. J.,Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows ( <i>Pimephales promelas</i> ): volume II.	<b>12</b>
<b>58126</b>	Acute (0-96 hour); Aquatic; Fish	Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. <i>Bulletin of Environmental Contamination and Toxicology</i> 20:344-352	<b>17</b>
<b>581262222</b>	Acute (0-96 hour); Aquatic; Fish	Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. <i>Bulletin of Environmental Contamination and Toxicology</i> 20:344-352	<b>20</b>
<b>68271</b>	Other; Aquatic; other South African clawed frog	Fort, D. J.,Stover, E. L.,Rayburn, J. R.,Hull, M.,Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using <i>Xenopus</i> . <i>Birth Defects Research, Part B: Developmental and Reproductive Toxicology</i> 13:35-45	<b>23</b>
<b>200570</b>	Acute (0-96 hour); Aquatic; Invertebrates	Sanchez-Fortun, S.,Sanz, F.,Santa-Maria, A.,Ros, J. M.,De Vicente, M. L.,Encinas, M. T.,Vinagre, E.,Barahona, M. V.. 1997. Acute sensitivity of three age classes of <i>Artemia salina</i> larvae to seven chlorinated solvents. <i>Bulletin of Environmental Contamination and Toxicology</i> 59:445-451	<b>28</b>
<b>660790</b>	Acute (0-96 hour); Aquatic; Plants	Brack, W.,Frank, H.. 1998. Chlorophyll a fluorescence: A tool for the investigation of toxic effects in the photosynthetic apparatus. <i>Ecotoxicology and Environmental Safety</i> 40:34-41	<b>32</b>
<b>661061</b>	Acute (0-96 hour); Aquatic; Plants	Brack, W.,Rottler, H.. 1994. Toxicity testing of highly volatile chemicals with green algae: A new assay. 1:223-228	<b>36</b>
<b>676758</b>	Acute (0-96 hour); Aquatic; Invertebrates	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> 43:149-157	<b>40</b>

<b>700434</b>	Other; Aquatic; other amphibian - wood frog and green frog	McDaniel, T.,Martin, P.,Ross, N.,Brown, S.,Lesage, S.,Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109	<b>43</b>
<b>700434</b>	Other; Aquatic; other amphibian - american toad	McDaniel, T.,Martin, P.,Ross, N.,Brown, S.,Lesage, S.,Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109	<b>50</b>
<b>700434</b>	Other; Aquatic; other amphibian - spotted salamder	McDaniel, T.,Martin, P.,Ross, N.,Brown, S.,Lesage, S.,Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109	<b>57</b>
<b>701994</b>	Other; Aquatic; other amphibian - frog	Fort, D.,Rayburn, J.,Deyoung, D.,Bantle, J.. 1991. Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug and Chemical Toxicology 14:143-160	<b>65</b>
<b>701995</b>	Other; Aquatic; other amphibian - frog	Fort, D.,Rogers, R.,Stover, E.,Finch, R.. 2001. Optimization of an exogenous metabolic activation system for FETAX. I. Post-isolation rat liver microsome mixtures. Drug and Chemical Toxicology 24:103-115	<b>72</b>
<b>707209</b>	Acute (0-96 hour); Aquatic; Invertebrates	Niederlehner, B.,Cairns, J.,Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. Ecotoxicology and Environmental Safety 39:136-146	<b>77</b>
<b>707209</b>	Other; Aquatic; Invertebrates	Niederlehner, B.,Cairns, J.,Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. Ecotoxicology and Environmental Safety 39:136-146	<b>81</b>
<b>1059985</b>	Acute (0-96 hour); Aquatic; Plants	Labra, M.,Mattia, F.,Bernasconi, M.,Bertacchi, D.,Grassi, F.,Bruni, I.,Citterio, S.. 2010. The Combined Toxic and Genotoxic Effects of Chromium and Volatile Organic Contaminants to Pseudokirchneriella subcapitata. Water, Air, and Soil Pollution 213:57-70	<b>86</b>
<b>1486051</b>	Acute (0-96 hour); Aquatic; Invertebrates	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	<b>89</b>
<b>1745587</b>	Other; Aquatic; Fish	Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.	<b>92</b>
<b>1745587</b>	Acute (0-96 hour); Aquatic; Plants	Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.	<b>96</b>
<b>2127844</b>	Acute (0-96 hour); Aquatic; Plants	Bacsi, I.,Toeroek, T.,B-Beres, V.,Toeroek, P.,Tothmeresz, B.,Nagy, A. S.,Vasas, G.. 2013. Laboratory and microcosm experiments testing the toxicity of chlorinated hydrocarbons on a cyanobacterium strain (Synechococcus PCC 6301) and on natural phytoplankton assemblages. Hydrobiologia 710:189-203	<b>101</b>

<b>2127941</b>	Acute (0-96 hour); Aquatic; Invertebrates	Dobaradaran, S.,Mahvi, A. H.,Nabizadeh, R.,Ramavandi, B.,Nazmara, S.,Zarei, S.. 2012. BIOASSAY COMPARISON OF TRICHLOROETHYLENE (TCE) TOXICITY ON DAPHNIA MAGNA (D. MAGNA) BEFORE AND AFTER ULTRASOUND AND PHOTOLYSIS PROCESSES. Fresenius Environmental Bulletin 21:1533-1538	<b>105</b>
<b>2128165</b>	Acute (0-96 hour); Aquatic; Plants	Lukavsky, J.,Furnadzheva, S.,Dittrt, F.. 2011. Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria. Bulletin of Environmental Contamination and Toxicology 86:226-231	<b>108</b>
<b>2298399</b>	Acute (0-96 hour); Aquatic; Fish	Smith, A. D.,Bharath, A.,Mallard, C.,Orr, D.,Smith, K.,Sutton, J. A.,Vukmanich, J.,McCarty, L. S.,Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102	<b>112</b>
<b>2298399</b>	Chronic (>21 days); Aquatic; Fish	Smith, A. D.,Bharath, A.,Mallard, C.,Orr, D.,Smith, K.,Sutton, J. A.,Vukmanich, J.,McCarty, L. S.,Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102	<b>115</b>
<b>2307041</b>	Other; Aquatic; other	Rayburn, D. J. Deyoung, J. A. Bantle, D. J. Fort, R. Mcnew. 1991. Altered developmental toxicity caused by three carrier solvents. Journal of Applied Toxicology 11:253-260	<b>118</b>
<b>2800252</b>	Other; Aquatic; Fish	Hayashi, M.,Ueda, T.,Uyeno, K.,Wada, K.,Kinae, N.,Saotome, K.,Tanaka, N.,Takai, A.,Sasaki, Y. F.,Asano, N.,Sofuni, T.,Ojima, Y.. 1998. Development of genotoxicity assay systems that use aquatic organisms. Mutation Research 399:125-133	<b>122</b>
<b>2801609</b>	Acute (0-96 hour); Aquatic; Plants	Ward, G. S.,Tolmsoff, A. J.,Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836	<b>125</b>
<b>2801609</b>	Acute (0-96 hour); Aquatic; Fish	Ward, G. S.,Tolmsoff, A. J.,Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836	<b>128</b>
<b>2801609</b>	Acute (0-96 hour); Aquatic; Invertebrates	Ward, G. S.,Tolmsoff, A. J.,Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836	<b>131</b>
<b>2803221</b>	Acute (0-96 hour); Aquatic; Fish	Dierickx, P. J.. 1993. Comparison between fish lethality data and the in vitro cytotoxicity of lipophilic solvents to cultured fish cells in a two-compartment model. Chemosphere 27:1511-1518	<b>134</b>
<b>3298076</b>	Acute (0-96 hour); Aquatic; Plants	Bacsi, I.,Gonda, S.,B-Beres, V.,Novak, Z.,Nagy, S. A.,Vasas, G.. 2015. Alterations of phytoplankton assemblages treated with chlorinated hydrocarbons: effects of dominant species sensitivity and initial diversity. Ecotoxicology 24:823-834	<b>138</b>

<b>3502953</b>	Other; Aquatic; Invertebrates	Houde, M.,Douville, M.,Gagnon, P.,Sproull, J.,Cloutier, F.. 2015. Exposure of <i>Daphnia magna</i> to trichloroethylene (TCE) and vinyl chloride (VC): evaluation of gene transcription, cellular activity, and life-history parameters. <i>Ecotoxicology and Environmental Safety</i> 116	<b>142</b>
<b>3546158</b>	Other; Aquatic; Invertebrates	Vidal, M.,Bassères, A.,Narbonne, J.. 2001. Potential biomarkers of trichloroethylene and toluene exposure in <i>Corbicula fluminea</i> . <i>Environmental Toxicology and Pharmacology</i> 9:87-97	<b>146</b>
<b>3617103</b>	Other; Aquatic; Plants	Ando, T.,Otsuka, S.,Nishiyama, M.,Senoo, K.,Watanabe, M. M.,Matsumoto, S.. 2003. Toxic Effects of Dichloromethane and Trichloroethylene on the Growth of Planktonic Green Algae, <i>Chlorella vulgaris</i> NIES227, <i>Selenastrum capricornutum</i> NIES35, and <i>Volvoxina steinii</i> NIES545. 18:43-46	<b>150</b>
<b>3617749</b>	Other; Aquatic; Invertebrates	Yoshioka, Y.,Ose, Y.,Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21	<b>153</b>
<b>3617749</b>	Acute (0-96 hour); Aquatic; Fish	Yoshioka, Y.,Ose, Y.,Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21	<b>157</b>
<b>3617749</b>	Acute (0-96 hour); Aquatic; Invertebrates	Yoshioka, Y.,Ose, Y.,Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21	<b>160</b>
<b>3617867</b>	Acute (0-96 hour); Aquatic; Plants	Tsai, K. P.,Chen, C. Y.. 2007. An Algal Toxicity Database of Organic Toxicants Derived by a Closed-System Technique. <i>Environmental Toxicology and Chemistry</i> 26:1931-1939	<b>163</b>
<b>3625489</b>	Other; Aquatic; Fish	Schell, J. D. J.. 1987. Interactions of Halogenated Hydrocarbon Mixtures in the Embryo of the Japanese Medaka ( <i>Oryzias latipes</i> ).	<b>166</b>
<b>3665276</b>	Acute (0-96 hour); Aquatic; Fish	Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow ( <i>Pimphales promelas</i> ). 24:3117-3127	<b>169</b>
<b>3665276</b>	Chronic (>21 days); Aquatic; Fish	Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow ( <i>Pimphales promelas</i> ). 24:3117-3127	<b>174</b>
<b>3683110</b>	Chronic (>21 days); Aquatic; Invertebrates	Leblanc, G. A.,Surprenant, D. C.. 1980. The Chronic Toxicity of 8 of the 65 Priority Pollutants to the Water Flea ( <i>Daphnia magna</i> ).	<b>180</b>

Study Citation: Leblanc, G. A.. 1980. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bulletin of Environmental Contamination and Toxicology 24:684-691  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 7508

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Chemical is identified by name.
	Metric 2: Test Substance Source	Low	× 1	3	All chemicals tested were purchased from commercial chemical suppliers, but the study does not specify what supplier provided TCE.
	Metric 3: Test Substance Purity	Low	× 1	3	Study reports a minimum purity of 80 percent for all chemicals tested, but does not specify what the purity is for TCE.
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	Negative control consisted of the same dilution water, test conditions, and test organisms, but containing no test substance or co-solvent. When appropriate a solvent control was also used.
	Metric 5: Negative Control Response	High	× 1	1	Mortality among water flea control populations never exceeded 10 percent in any test.
	Metric 6: Randomized Allocation	High	× 1	1	Five daphnids were randomly placed in each 150 mL test solution within 30 minutes of the solution preparation.
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	Low	× 2	6	It appears the volatility of TCE might have been taken into account in the test methods, but it's unclear. The study reports that, generally, "The tests were also conducted in unreplicated 500 mL solutions containing 15 daphnids if dividing the solution into triplicate test vessels presented a risk of the loss of the test substance through volatilization or if vapors of the substance posed a high health risk to the investigators. In addition, these vessels were covered with plastic wrap secured with an elastic band."
	Metric 8: Consistency of Exposure Administration	Medium	× 1	2	Only minor uncertainties

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	7508				

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	Measurements were not reported and the test substance is volatile.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	Exposure duration is 48 hours which is OECD TG 202 recommended. Only one dose for the 48 hour period as this is an acute study.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	5-8 exposure groups were used for each chemical. No range finding was conducted to determine an appropriate exposure, but it appears they were appropriate enough to establish an LD50.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	<i>Daphnia magna</i> less than 24 hours old were used in these toxicity tests from laboratory stocks cultured at EG&G, Bionomics.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Study didn't report whether test organisms were acclimatized.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	It appears there were 15 daphnia in each test concentration for TCE and no replicates to avoid losing TCE to vitalization. OECD TG 202 recommends at least 20 and separated into 4 different vessels.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Ph, dissolved O <sub>2</sub> , temp, and 2ml per organism were all appropriate for <i>daphnia magna</i> according to OECD TG 202.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Mortality data from these toxicity tests were used to calculate a median lethal concentration and its 95 percent confidence limits based on nominal concentrations.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	

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 Hero ID: 7508

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	The LC50's and 95 percent confidence limits were calculated utilizing a moving average angle method (Stephan, personal communication) when possible. With the the moving average angle method, the nominal test concentrations were transformed to logarithms and the corresponding percentage mortalities to angles. Each group of three successive angles was then averaged and the LC50 was estimated by linear interpolation between the successive concentrations whose average angles bracketed 45 degrees. When test data did not meet the moving average angle method requirements, the LC50's were estimated by probit analysis by converting the concentrations to logarithms and percentage mortalities to probits and then calculating a least squares linear regression analysis. Finally, if the data did not permit a probit analysis, then a binomial probability analysis was performed on these data. Calculations were performed with a Hewlett-Packard Hodel 9815A calculator programed to scan the data base and to select the most appropriate of the three methods described above for the calculation of an LC50. If no mortality was observed in water flea populations exposed to a nominal concentration of approximately 500 mg/L, no additional concentrations were tested and the LC50 was estimated to be greater than the highest nominal concentration tested (>530 mg/L). LC50's and 95 percent confidence limits were reported after rounding to 2 significant figures. No correction was made for control mortality.
	Metric 22: Reporting of Data	Medium	× 2	4	Data for most but not all outcomes by study group were reported but these minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes reported

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Overall Quality Determination <sup>‡</sup>		High		1.6	
Extracted		Yes			

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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

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*).  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 18050

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Chemical identified as 1,1,2-trichloroethylene
	Metric 2: Test Substance Source	High	× 1	1	
	Metric 3: Test Substance Purity	Low	× 1	3	Each compound was received in individual, sealed vials from, New England Nuclear, Boston, Massachusetts, and held under refrigerated conditions until used. No purity of test chemical was reported. Liquid gas chromatography was performed during the experiment and purity of the chemical could be determined then, but it wasn't reported in the paper.
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	Concentrations of TCE in fish tissue were measured in controls and compared to test species. Method for allocation was not reported.
	Metric 5: Negative Control Response	High	× 1	1	
	Metric 6: Randomized Allocation	Low	× 1	3	
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	High	× 2	2	"Steps were taken to minimize vitalization. Each test aquaria was a closed system for the volatile organics. Each fish was wrapped in Para film" at each sampling to minimize the volatilization of each chemical from the fish tissue during the combustion process."
	Metric 8: Consistency of Exposure Administration	High	× 1	1	

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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*).  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 18050

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	Half-life was defined as the period of time required for one-half of the mean chemical residues measured in fish tissues at equilibrium or at the end of the exposure period to be eliminated following termination of exposure. The specific activity of the carbon 14-labeled stock solution was measured radio-metrically prior to test initiation. During each test exposure, representative water and fish samples, including samples from control aquaria, were collected on days 0, 1, 2, 4, 7, 10, 14, 21 and 28 (if exposure continued for the entire 28 days) and analyzed for residues or metal concentrations as appropriate. Fish were sampled on days 1, 2, 4 and 7 to estimate the half-life of chemical residues in tissues.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	Exposure was for 14 days. OECD recommends 28 but shorter is ok if it takes less time for the fish residues to reach a steady state in relation to the chemical in the water. Authors mentioned that this was the case. Study used a continuous flow through system for the 14 days.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	"The levels of exposure were selected on the basis of acute toxicity data generated at Bionomics and were intended to be sublethal during the continuous exposure period."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	Medium	× 2	4	Minor reservations about the source of fish. Three populations of bluegill sunfish ( <i>Lepomis macrochirus</i> ) were obtained from a commercial fish farmer in Connecticut. May not all be the same age, but length and weight was documented, and age may not be a big factor in determining BCF.
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	× 1	2	Fish were maintained in the holding facilities for a minimum of 30 days prior to the initiation of the study. Minor uncertainties in the details provided.

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Data Type:	Other; Aquatic; Fish				
Hero ID:	18050				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Study started with 100 organisms per exposure group, and took fish out 5 fish on each sampling day. OECD recommends having enough to remove at least 4. Unsure the number of replicates.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Recommended temp for blue gill is 20-25 degrees C and this study was conducted at 16 degrees C which could have lowered metabolism in fish.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Unacceptable	× 2	8	BCFs, which measure bioconcentration, were out of scope for the environmental hazard assessment portion of the risk evaluation. These were assessed elsewhere in the risk evaluation. Also, in this study BCFs and half-lives were reported for each of the chemicals. Assessment was not as sensitive as it should be for calculating a BCF - OECD recommends noting if both sexes are used, differences in growth and lipid content between sexes should be documented to be non-significant before the start of the exposure, in particular if it is anticipated that pooling of male and female fish will be necessary to ensure detectable substance concentrations and/or lipid content. This was not noted.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	OECD recommends noting If both sexes are used, differences in growth and lipid content between sexes should be documented to be non-significant before the start of the exposure, in particular if it is anticipated that pooling of male and female fish will be necessary to ensure detectable substance concentrations and/or lipid content. This was not noted.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
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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> ).				
Data Type:	Other; Aquatic; Fish				
Hero ID:	18050				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 21: Statistical Methods	High	× 1	1	A steady-state bioconcentration factor (BCF) was calculated as the quotient of the mean chemical concentration measured in fish tissues during equilibrium divided by the mean measured chemical concentration in water during the entire exposure period (Table III).
	Metric 22: Reporting of Data	Medium	× 2	4	Not all regressions, lipid content, and weights were reported, but BCFs and half lives were reported for all chemicals.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes.
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		Yes			

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Buccafusco, R. J.,Ells, S. J.,Leblanc, G. A.. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). Bulletin of Environmental Contamination and Toxicology 26:446-452  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 18064

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	
Metric 2:	Test Substance Source	Low	× 1	3	The study says all chemicals tested were purchased from commercial chemical suppliers, but does not specify where TCE came from.
Metric 3:	Test Substance Purity	Low	× 1	3	Study reports a minimum purity of 80 percent for all chemicals tested, but does not specify what the purity is for TCE.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Both negative and solvent controls were used.
Metric 5:	Negative Control Response	Low	× 1	3	There were many chemicals tested and do not give details about negative control response, although it says control mortality was recorded.
Metric 6:	Randomized Allocation	High	× 1	1	"Ten fish were randomly selected from a test population and added to each test jar within 30 min after the addition of the test chemical or stock solution."
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	Volatile chemicals were capped, but it is unclear whether headspace was minimized in the jars and with the jars capped could have had low DO content.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations were used and were not measured. TCE is volatile.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	Exposure is 96 hours which is OECD TG 203 recommended.

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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 26:446-452				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	18064				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Study says that the test was conducted according to EPA's "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" which says for static tests you must have 10 organisms in each treatment divided into at least two test chambers; not sure how they got the exposure concentrations used of what the exposure concentrations were.
	Metric 12: Testing at or Below Solubility Limit	Low	× 1	3	Test substance concentration was not reported, and it was reported that some concentration there was undissolved chemical.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Test animals utilized were young of the year bluegill ( <i>L. macrochirus</i> ) obtained from commercial fish suppliers within the continental United States.
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	While it was reported that there was a 48 hour time where fish were observed and not used if had >3 percent mortality, it was not reported whether they were held for 12 days in the lab before they are used for testing.
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Minor uncertainties around number of organisms used.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Minor uncertainties around housing conditions (headspace in jar, DO concs).
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	24 and 96 hour LC50s
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	Study did not provide enough information to allow a comparison of environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Study did not provide enough information about health outcomes of each study group.
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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 26:446-452				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	18064				

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	Not clear what method was used for TCE: "The LC50s and 95 percent confidence intervals were calculated, where possible, by the moving average angle method (HARRIS 1959). The nominal test concentrations were transformed to logarithms and corresponding percentage mortalities to angles. Each group of these successive angles was then averaged and the LC50 was estimated by linear interpolation between the successive concentrations whole average angles bracketed 45". When the test data did not meet Harris' method requirements, the LC50s were calculated by the log probit method, a modification of the LITCHFIELD + WILCOXON (1949) method."
	Metric 22: Reporting of Data	Low	× 2	6	The data for the static test were not presented in full, and no information was reported for controls.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There were no unexpected outcomes for TCE in this paper.
Overall Quality Determination <sup>‡</sup>		Medium		2.0	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Geiger, D. L., Northcott, C. E., Call, D. J., Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): volume II.  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 32169

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Trichloroethylene identified by name, CAS, formula, and molecular weight.
Metric 2:	Test Substance Source	High	× 1	1	Aldrich Chemical Co
Metric 3:	Test Substance Purity	Medium	× 1	2	Purity reported at 98 percent, no mention of what impurities were.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Two controls reported. Unsure what kind (water or solvent?)
Metric 5:	Negative Control Response	High	× 1	1	Response of control was reported.
Metric 6:	Randomized Allocation	High	× 1	1	At the start of a test, individual fish were removed from the common pool of fish with a net and distributed at random among the exposure chambers.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Flow through system used using cycling proportional diluters with duplicate tanks for each test conc. It's unclear exactly what system was used for TCE because the description at the beginning of the paper is non-specific. It seems like the following system was used: The electronic diluter was used for expensive and volatile chemicals or when acute toxicity was very close to water solubility. Another form of a liquid-liquid equilibrator was constructed from a 2.8 L culture flask atop a magnetic stirrer. A pump forced lake water into this closed system which contained a layer of the chemical.
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration was reported but it's unclear what type of administration applies to what chemicals.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Test concentrations were measured by gas-liquid chromatography.

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Study Citation: Geiger, D. L., Northcott, C. E., Call, D. J., Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): volume II.  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 32169

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	High	× 1	1	96 hour exposure using flow-through method for TCE
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Five exposure concentrations were used, and measured concentrations were used, but it's unclear how exactly an appropriate conc was determined. No limit test is mentioned, only that test mean concentrations were usually >60 percent of saturation. The toxicant concentrations were 100, 80, 60, 40, and 20 percent of the toxicant stock. In cases where test chemical quantities were limited, toxicant concentrations were condensed to 100, 66, and 33 percent of the toxicant stock.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Measured conc were reported and are below solubility.
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	High	× 2	2	Fathead minnows used in this test.

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Study Citation:	Geiger, D. L., Northcott, C. E., Call, D. J., Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows ( <i>Pimephales promelas</i> ): volume II.				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	32169				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	Only minor uncertainties. Study reports, "Fathead minnows used in the tests were cultured at the U.S. EPA Environmental Research Laboratory-Duluth and the University of Wisconsin-Superior campus. Adults were held at 25°C in flowing water with a 16 hr light-controlled photo-period and fed frozen adult brine shrimp ( <i>Artemia</i> sp.). They were provided with asbestos pipes (cut in half longitudinally) as spawning substrates, where naturally spawned and fertilized embryos attached to the underside. The substrates, with intact embryos, were removed daily and placed in another 25C bath where hatching occurred; however the spawning substrates were removed just prior to hatching at the UW-Superior culture unit, then placed in a rearing bath. For tests conducted in 1977-1982, newly hatched larvae from the stock culture unit were reared in a system similar to the exposure systems at a temperature of 25C. Tests conducted following 1982 used fish that had been reared in flow-through tanks in the lab 1s culture unit. Larvae were fed 40-48 hr old brine shrimp nauplii (Bio-Marine Research, Inc., Hawthorne, CA) in excess two times daily (once on week-end days). Embryos and larvae were cultured in water from the same source as used in the exposures to the test chemicals. Fish that were approximately 28-34 days old were used in the toxicity tests." Note, it is acceptable to use asbestos pipes for spawning purposes.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Number of test organisms was not reported for studies prior to 1982. The TCE test was in 1979.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Only minor uncertainties. The temperature is appropriate for fathead minnows according to OECD guidelines (OECD TG 203).
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	An LC50 was derived.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Consistent assessment for outcomes in each study group.

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Study Citation:	Geiger, D. L., Northcott, C. E., Call, D. J., Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows ( <i>Pimephales promelas</i> ): volume II.				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	32169				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences between study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition or health impacts unrelated to exposure were not reported for each study group, because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	The estimated LC50 (lethal concentration causing 50 percent mortality of the fish) and EC50 (effect concentration causing 50 percent of the fish to show an effect) with corresponding 95 percent confidence intervals were calculated using the corrected averages of the analyzed tank concentrations and the Trimmed Spearman-Kärber Method (Hamilton et al., 1977). In cases of duplicate exposures, the effect and mortality data were pooled, resulting in a single estimate of LC50 and EC50 per test. The EC50s are based upon loss of equilibrium manifested by the fish's inability to maintain an upright position when swimming. Calculations were made for 96 hr of exposure and also for intermediate exposure times. The mean toxicant concentrations used in the calculations were corrected for analytical recoveries of spiked water samples. Some LC50 data may vary slightly from previously published literature due to the inclusion of a spike recovery factor in this volume.
	Metric 22: Reporting of Data	High	× 2	2	Each exposure group results were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes were reported.
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			
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Study Citation: Geiger, D. L., Northcott, C. E., Call, D. J., Brooke, L. T. eds. 1985. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*): volume II.  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 32169

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 58126

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	trichloroethylene identified by name
Metric 2:	Test Substance Source	Medium	× 1	2	Authors work for chemical company that produces TCE.
Metric 3:	Test Substance Purity	Low	× 1	3	Not indicated, but because TCE is a common solvent produced by the company, assuming at minimum technical grade.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	
Metric 5:	Negative Control Response	Low	× 1	3	Not indicated, only LC50 for TCE reported., but no deviances from test guideline [COMMITTEE ON METHODS FOR TOXICITY TESTS WITH-AQUATIC ORGANISMS (1975)] indicated.
Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not indicated, but followed test guidelines.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Covered with plastic wrap, but no indication that headspace minimized.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	No measurements were made in the static test and this chemical is very volatile. It was mentioned that containers were covered but there was no mention of headspace minimizing. "In the static water fish toxicity tests the nominal concentration, or that amount of chemical initially added to the test chambers, was used to calculate the LC 10, LC 50, and LC 90 toxicity values."
Metric 10:	Exposure Duration and Frequency	High	× 1	1	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Test concentrations not provided.

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Study Citation:	Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	58126				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Low	× 2	6	Fathead minnow is a well known lab species; but from a bait shop, rather than cultured under controlled conditions.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Not indicated, but based on use of test guideline, suggests adequate number for statistical analysis.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	High	× 2	2	
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			
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Study Citation: Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 58126

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 581262222

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	
Metric 2:	Test Substance Source	Medium	× 1	2	Authors work for chemical company that produces TCE.
Metric 3:	Test Substance Purity	Low	× 1	3	Not indicated, but because TCE is a common solvent produced by the company, assuming at minimum technical grade.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Methyl or ethyl alcohol was used as the carrier solvent for all compounds except methylene chloride. A control containing the same amount of alcohol that is present in the highest chemical concentration and a Lake Huron water control were included in each test series.
Metric 5:	Negative Control Response	Low	× 1	3	Not indicated, only LC50 for TCE reported., but no deviations from test guideline [COMMITTEE ON METHODS FOR TOXICITY TESTS WITH-AQUATIC ORGANISMS (1975)] indicated.
Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not indicated, but followed test guidelines.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	Covered with plastic wrap, but no indication that headspace minimized.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Gas chromatography used to analyze test concentrations daily.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Test concentrations not provided.

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Study Citation:	Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	581262222				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Low	× 2	6	Fathead minnow is a well known lab species; but from a bait shop, rather than cultured under controlled conditions.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Not indicated, but based on use of test guideline, suggests adequate number for statistical analysis.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	High	× 2	2	
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			
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Study Citation: Alexander, H. C.,McCarty, W. M.,Bartlett, E. A.. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 58126222

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Fort, D. J., Stover, E. L., Rayburn, J. R., Hull, M., Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using *Xenopus*. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 13:35-45  
 Data Type: Other; Aquatic; other South African clawed frog  
 Hero ID: 68271

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by its name trichloroethylene.
Metric 2:	Test Substance Source	High	× 1	1	"Trichloroethylene and cyclohexene oxide were obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin."
Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade were not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	"Ten to sixteen concentrations were tested in duplicate. Four separate dishes of 20 embryos each were exposed to FETAX solution and designated FETAX solution controls. Controls, including FETAX solution, 1 percent v/v DMSO, uninhibited MAS (with and without DMSO), each inhibited MAS (with and without DMSO), activated acetylhydrazide (FETAX reference proteratogen), and unactivated toxicant, were tested simultaneously with each experiment. All control treatments received antibiotics, as well."
Metric 5:	Negative Control Response	High	× 1	1	"In each of the experiments conducted, the FETAX solution, 1 percent v/v DMSO, and MAS or MAS with 1 percent v/v DMSO, control mortality and malformation rates were less than or equal to 5 percent. Mortality and malformation rates for the inhibited MAS failed to exceed 6 percent. Embryos exposed to 3.0 g/l acetylhydrazide and the intact MAS exhibited mortality and deformity rates of greater than or equal to 90 percent and 100 percent, respectively."
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization					
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Study Citation: Fort, D. J., Stover, E. L., Rayburn, J. R., Hull, M., Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using *Xenopus*. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 13:35-45  
 Data Type: Other; Aquatic; other South African clawed frog  
 Hero ID: 68271

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	A static renewal test was used for this assay and closed dishes were used which is important given TCE's volatility. "For experiments conducted without the MAS, groups of 20 embryos were placed in covered 60 mm plastic Petri dishes (Fisher Scientific, Houston, Texas) with varying constant concentrations of trichloroethylene, dichloroacetic acid, trichloroacetic acid, trichloroethanol or oxalic acid."
Metric 8:	Consistency of Exposure Administration	High	× 1	1	"Trichloroethylene was dissolved in 1 percent v/v dimethylsulfoxide (DMSO) in FETAX solution. Ten to sixteen concentrations were tested in duplicate. Four separate dishes of 20 embryos each were exposed to FETAX solution and designated FETAX solution controls. Each treatment vessel contained a total of 8 ml of solution. Tests conducted with the MAS or inhibited MAS were also performed in duplicate with 20 embryos exposed per replicate concentration. Each metabolically activated treatment received 0.1 ml/dish of N-nitrosodimethylamine activity 1 a NADPH generating system, and a penicillin-streptomycin mixture to control bacterial contamination. For each treatment, 8 to 14 concentrations were tested. Controls, including FETAX solution, 1 percent v/v DMSO, uninhibited MAS (with and without DMSO), each inhibited MAS (with and without DMSO), activated acetylthiocholine (FETAX reference proteratogen) [ 12], and unactivated toxicant, were tested simultaneously with each experiment. All control treatments received antibiotics, as well. One range-finding and two definitive concentration-response experiments were conducted with and without the MAS or inhibited MAS. The pH of each of the stock solutions was adjusted to 7.0 with NaOH. Embryos were cultured at 23 °C. All solutions were changed every 24 h of the 4-day test, dead embryos removed, and fresh solutions added. Following 96 h of exposure (stage 46 embryos), embryos were fixed in 0.7 percent formalin (pH 7.0), and the number of live malformed embryos were ascertained using a dissecting microscope."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Study Citation: Fort, D. J., Stover, E. L., Rayburn, J. R., Hull, M., Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using <i>Xenopus</i> . Birth Defects Research, Part B: Developmental and Reproductive Toxicology 13:35-45					
Data Type: Other; Aquatic; other South African clawed frog					
Hero ID: 68271					
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	Study does not report whether measurements were taken, but it does say that it was a static renewal test and that each petri dish had varying constant concentrations of TCE.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	96 hours of exposure and which is recommended for African clawed frog tadpoles for acute tests and fresh solution was added every 24 hours.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	It was mentioned that a range finding test was conducted prior to the assay, but not mentioned how the spacing of the conc were decided on.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	EC50s were far below the solubility for TCE.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	<i>Xenopus laevis</i> was used and is the recommended species for these tests according to EPA's test guidance 890.2300.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Unable to fully determine from this study because it reports that "Xenopus adult care, breeding, and embryo collection were performed as described by Dawson and Ban tie [ 17]."
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	20 animals per dish were tested which is recommended in EPA guidelines, but only two replicates were used where as guidelines recommend 3 for exposure groups and 4 for controls.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	EPA guidelines (OPPTS 890.1100) recommend 4 " 10 L (10 - 15 cm minimum water depth) /glass or stainless steel, and study reports only 8 ml of solution.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	A 96 hour EC50 and LC50 were determined
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol across study groups.
Domain 6: Confounding / Variable Control					
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Study Citation:	Fort, D. J., Stover, E. L., Rayburn, J. R., Hull, M., Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using <i>Xenopus</i> . Birth Defects Research, Part B: Developmental and Reproductive Toxicology 13:35-45				
Data Type:	Other; Aquatic; other South African clawed frog				
Hero ID:	68271				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and health outcomes unrelated to exposure were not reported for each study group. (Table 2 and 3 show percent responding out of the total number of organisms for each exposure group, but not attrition, or other health outcomes.)
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	"Litchfield-Wilcoxon probit analysis [ 19] was used to determine the 96 h median lethal (LC50) and teratogenic (EC50) concentrations of two pooled definitive concentration-response tests. The 95 percent confidence intervals were calculated as well. A teratogenic index (TI) was calculated by taking the ratio of the 96 h LC50 value to the 96 h EC50 (malformation) value as a means of assessing teratogenic potential [ 17]. Ninety-five percent fiducial intervals for the TI values were calculated by the method of Finney [20]. Head-tail length of surviving embryos was measured as an index of growth using an IBM-AT compatible computer and Sigma Scan (Jandel Scientific, Corte Madra, CA) digitizing software. The length data were then used to calculate a minimum concentration to inhibit growth (MCIG) value for each experiment using the t-test (P <0.05)."
	Metric 22: Reporting of Data	High	× 2	2	Data on exposure related findings were presented for each treatment and control group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There was an explanation of why the authors thought the controls died in the MAS activated group.
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			
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Study Citation: Fort, D. J., Stover, E. L., Rayburn, J. R., Hull, M., Bantle, J. A.. 1993. Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using Xenopus. Birth Defects Research, Part B: Developmental and Reproductive Toxicology 13:35-45  
 Data Type: Other; Aquatic; other South African clawed frog  
 Hero ID: 68271

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation:	Sanchez-Fortun, S., Sanz, F., Santa-Maria, A., Ros, J. M., De Vicente, M. L., Encinas, M. T., Vinagre, E., Barahona, M. V.. 1997. Acute sensitivity of three age classes of <i>Artemia salina</i> larvae to seven chlorinated solvents. <i>Bulletin of Environmental Contamination and Toxicology</i> 59:445-451				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	200570				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as 1,1,1-trichloroethylene
Metric 2:	Test Substance Source	High	× 1	1	Ethanol and analysis grade chlorinated solvents were obtained from Sigma Chemical Company (St. Louis, USA).
Metric 3:	Test Substance Purity	Medium	× 1	2	Purity not provided but authors note it is analytical grade
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Appropriate control systems were included in each experiment. One of the untreated controls was exposed to ethanol which was used to solubilize the chlorinated solvents; in all cases the concentration of ethanol never exceeded a final concentration of 1%, which was non-toxic. The other control consisted of a single synthetic seawater. The plates were then incubated at 25°C in the dark.
Metric 5:	Negative Control Response	Medium	× 1	2	Control response not reported, however, this is not expected to affect results for the seawater control. Typically multi-chemical tests will only report control results if significant ( <i>i.e.</i> , > 10 percent mortality).
Metric 6:	Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Nominal concentrations used without steps to reduce volatilization of TCE.
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details were not provided.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations with no analytical monitoring reduces confidence in study results for methylene chloride, but a trend is apparent when compared across the solvents tested that informs the relative toxicity of TCE.
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Study Citation: Sanchez-Fortun, S., Sanz, F., Santa-Maria, A., Ros, J. M., De Vicente, M. L., Encinas, M. T., Vinagre, E., Barahona, M. V.. 1997. Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. *Bulletin of Environmental Contamination and Toxicology* 59:445-451

Data Type: Acute (0-96 hour); Aquatic; Invertebrates

Hero ID: 200570

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	Medium	× 1	2	24 hours
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Study does not provide exposure concentrations, but paper indicates that "Each solvent concentration was set in sextuplicate" suggesting six exposure concentrations were used for TCE. LC50/EC50s were determined indicating exposure concentrations sufficiently spaced.
	Metric 12: Testing at or Below Solubility Limit	Medium	× 1	2	The range of concentrations tested and used to derive the LC50s were not reported.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	"The method of Persoone et al. (1989) to obtain <i>Artemia salina</i> for the test was applied and modified according to the following procedure. For this purpose, the encysted brine shrimp of species <i>Artemia salina</i> were obtained from 100 mg of eggs. They were hydrated in distilled water at 4°C for 12 hr, followed by washing to separate the cysts that sink from those that float. The cysts that sank were collected on a Büchner funnel and washed with cold distilled water, followed by synthetic seawater. Synthetic seawater was prepared by mixing 35 " of Synthetica sea salt (Waterlife Research Ltd., England), with distilled and deionized (Milli-Q) water, stirring for 24 hr with suitable aeration and successive filtration with thick cellulose filters. They were then incubated in a graduated glass cylinder for about 24 hr at 25°C, with continuous side illumination (15-W fluorescent lamp), in 100 mL of synthetic seawater, at pH 8.6, and with a slight aeration maintained by a small tube in contact with the bottom of the cylinder. The nauplii produced were aspirated with Pasteur pipettes and transferred to two glass flasks containing 200 mL of the synthetic seawater, and maintained for another 24- or 48-hr."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	10 animals and four replicates per TCE concentration tested.

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Study Citation:	Sanchez-Fortun, S., Sanz, F., Santa-Maria, A., Ros, J. M., De Vicente, M. L., Encinas, M. T., Vinagre, E., Barahona, M. V.. 1997. Acute sensitivity of three age classes of <i>Artemia salina</i> larvae to seven chlorinated solvents. <i>Bulletin of Environmental Contamination and Toxicology</i> 59:445-451				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	200570				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Minor uncertainties regarding test conditions because they were not reported, and control response was not reported.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Medium	× 2	4	Details of the outcome assessment were not provided.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	× 2	4	The study did not provide enough information to allow a comparison of environmental conditions .
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes unrelated to exposure (i.e. controls) not reported, but not expected to affect interpretation of results.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	"The 24 hr-LC50 values, with 95 percent confidence limits, were calculated according to Litchfield and Wilcoxon method (1949) implemented in the PharmacologicCalculation System (PCS version 4.0, New York). These values were subjected to a two-way analysis of variance with replication within the subgroups (ANOVA), followed by post hoc contrast with Newman-Keuls Test."
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings not reported for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination <sup>‡</sup>		Medium	→ Low	1.8	This study used nominal concentrations without analytical measurement. The study did not report any effort to reduce volatilization of TCE during testing which could have substantial impacts on the results.
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Study Citation: Sanchez-Fortun, S., Sanz, F., Santa-Maria, A., Ros, J. M., De Vicente, M. L., Encinas, M. T., Vinagre, E., Barahona, M. V.. 1997. Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. *Bulletin of Environmental Contamination and Toxicology* 59:445-451  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 200570

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Brack, W., Frank, H.. 1998. Chlorophyll a fluorescence: A tool for the investigation of toxic effects in the photosynthetic apparatus. Ecotoxicology and Environmental Safety 40:34-41				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	660790				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Test substance was identified by name (Trichloroethylene)
	Metric 2: Test Substance Source	Low	× 1	3	Source was not identified.
	Metric 3: Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	Negative controls in distilled water were used.
	Metric 5: Negative Control Response	Low	× 1	3	Control response not reported.
	Metric 6: Randomized Allocation	Low	× 1	3	It was not reported whether there was random allocation to test groups
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	High	× 2	2	The volatility of chemicals was taken into consideration. The tubes were gas-tight sealed by using screw caps with Teflon-lined butyl rubber septa and continuously shaken for 2 h at a temperature of 20 C.
	Metric 8: Consistency of Exposure Administration	Low	× 1	3	Details of exposure for each study group were not reported. the study did say that " Aliquots of 5mL of the cell suspension were taken from the turbidostat and diluted in 10-mL brown glass tubes with the same volume of an aqueous solution of the chemical being tested. The tubes were gas-tight sealed by using screw caps with Teflon-lined butyl rubber septa and continuously shaken for 2 h at a temperature of 20°C. With this procedure, nonvolatile and volatile compounds could be tested. During incubation, light was excluded to prevent CO2 consumption by the algae and to avoid CO2 deficiency during incubation."
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether exposure concentration were measured or not.
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Study Citation: Brack, W., Frank, H.. 1998. Chlorophyll a fluorescence: A tool for the investigation of toxic effects in the photosynthetic apparatus. Ecotoxicology and Environmental Safety 40:34-41  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 660790

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	Medium	× 1	2	Exposure duration is not standard (600 seconds), but could be acceptable for what is being measured (fluorescence).
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	Unclear how many exposure groups or what the exposure levels were for TCE.
	Metric 12: Testing at or Below Solubility Limit	Low	× 1	3	Unknown exactly what conc were tested but the toxicity threshold is well below the high solubility of TCE.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Green algae ( <i>Chlamydomonas reinhardtii</i> )
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	× 1	2	Some acclimatization conducted with some minor uncertainties about pretreatment. The study says, "Green algae of the species <i>Chlamydomonas reinhardtii</i> [strain 11-32a SAG(#), according to Schlosser (1982)] were cultivated in a nutrient solution for unicellular algae (Kuhl and Lorenzen, 1964) in a turbidostat; use of the turbidostat provides exponentially growing cell suspensions of a constant density and physiological state by dilution with fresh medium controlled by a photoelectric cell. The algae were illuminated continuously by four cool white fluorescent tubes (4]10 W) aerated, and maintained at a temperature of 20°C. The cultures were kept at a density of 2]106 cells/mL for 2 weeks. The doubling time in the turbidostat was about 13 h."
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Not reported
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Teflon containers that were sealed
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Medium	× 2	4	For TCE a 600 second EC5 was reported of 13 (F'0/F0)
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	Details of the assessment protocol were not reported for each study group

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Study Citation:	Brack, W., Frank, H.. 1998. Chlorophyll a fluorescence: A tool for the investigation of toxic effects in the photosynthetic apparatus. Ecotoxicology and Environmental Safety 40:34-41				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	660790				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	Not enough information provided to allow a comparison of environmental conditions between study groups for TCE.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition from controls were not reported for each chemical explicitly but it was mentioned that the test concentrations were cored to the controls. "Toxicity thresholds (TTs) are defined as concentrations that reduce or increase at least one of the Fluorescence parameters for more than the threefold value of the maximum of standard deviations of the controls. A TT is attained when the measurement deviates by 3, 5, 10, or 20 percent from the respective control value, depending on the reproducibility of the particular parameter. The TTs of the tested chemicals, calculated by linear extrapolation between the highest concentration without significant effect and the lowest concentration with it, are presented in Table 3."
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	F'0/F0=EC05 and "Toxicity thresholds (TTs) are defined as concentrations that reduce or increase at least one of the Fluorescence parameters for more than the threefold value of the maximum of standard deviations of the controls. A TT is attained when the measurement deviates by 3, 5, 10, or 20 percent from the respective control value, depending on the reproducibility of the particular parameter. The TTs of the tested chemicals, calculated by linear extrapolation between the highest concentration without significant effect and the lowest concentration with it, are presented in Table 3."
	Metric 22: Reporting of Data	Low	× 2	6	The EC05 was reported to TCE but not much other detail was reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
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Study Citation: Brack, W., Frank, H.. 1998. Chlorophyll a fluorescence: A tool for the investigation of toxic effects in the photosynthetic apparatus. Ecotoxicology and Environmental Safety 40:34-41  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 660790

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Extracted		No			

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Brack, W., Rottler, H.. 1994. Toxicity testing of highly volatile chemicals with green algae: A new assay. 1:223-228  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 661061

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name.
Metric 2:	Test Substance Source	High	× 1	1	Authors identified Merck as the source of the test substance.
Metric 3:	Test Substance Purity	High	× 1	1	Authors reported greater than 99 percent purity.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	"Each test series contained three controls without toxicant and two controls with 0.8 mg/L Cu <sup>2+</sup> (CuSO <sub>4</sub> ). This concentration reduces algal growth to 50 percent and is used to check normal sensitivity of the organisms."
Metric 5:	Negative Control Response	Low	× 1	3	The biological responses of the negative control groups were not reported.
Metric 6:	Randomized Allocation	Low	× 1	3	It was not reported whether there was random placement of flasks.
Domain 3: Exposure Characterization					
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Study Citation: Brack, W., Rottler, H.. 1994. Toxicity testing of highly volatile chemicals with green algae: A new assay. 1:223-228  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 661061

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The purpose of the test was to determine a way of doing algae tests with volatile chemicals, as the OECD guidelines recommends using a permeable stopper in the flask to allow CO <sub>2</sub> to pass through so as not to impede algae growth. However with volatile chemicals this is not possible because of loss of test substance through vitalization. Therefore in test, they used a closed system that still provided a source of CO <sub>2</sub> for the algae. Authors reported, "Deviations between the duplicates, extracted from the same test culture were less than 5 percent . To estimate recovery of this analytical method, 20 mL headspace vials were filled completely with water or alga suspension. The vials were sealed gas"tight with septa. Gravimetrically defined amounts of the volatile chlorinated hydrocarbons were injected via syringe through the Septa into the liquids and dissolved. From these solutions samples were taken and extracted as explained above. Recovery of the method amounted to 90 % S percent and was independent from cell density."
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Concentrations were measured at the end of the test. Authors stated, "Sampling for measurement of toxicant concentration was possible without opening the vessel through septa with screw caps (- Fig. 1)." and "Measurements at the beginning and at the end of the assays showed no significant differences in chemical concentrations. Therefore, samples for analysis were taken at the end of the assay in order to avoid leaks in the septa, which could allow evaporation during the test period .
Metric 10:	Exposure Duration and Frequency	High	× 1	1	The test was 72 hours in duration, which is recommended by OECD Guideline 201.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Test concentrations are reported in figure 3 and show a dose response for growth inhibition. The figure shows at least 5 concentrations tested which is recommended by OECD Guideline 201.

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Study Citation: Brack, W., Rottler, H.. 1994. Toxicity testing of highly volatile chemicals with green algae: A new assay. 1:223-228					
Data Type: Acute (0-96 hour); Aquatic; Plants					
Hero ID: 661061					
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	The test conc for TCE shown in figure 3 (highest conc is <100 mg/l) are well below TCE's solubility level of 999-1472 mg/l.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	This is not a commonly used algal species. Not a TG species. Test used unicellular freshwater green alga <i>Chlamydomonas rethardtii</i> (strain number 11 -32a SAG) from the University of Gottingen, Germany.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Pretreatment conditions included, "Precultures and test cultures were grown in the medium for unicellular algae according to KUfL (1962) (Table 2). Incubation of all cultures was done in a Orbital Incubator (Gallenkamp). The cultures were shaken permanently with a frequency of 120 rpm. They were illuminated from above with 130 "E/m <sup>2</sup> s without light dark cycle. The photosynthetically effective light was determined with a Quantum Sensor from Licor Inc. The temperature was maintained at 20 " 1 0 -c."
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Two replicates per test concentration were reported. OECD Guideline 201 states the test should include three replicates, but if determination of a NOEC is not required, the test may be altered to increase the number of concentrations and reduce the number of replicates per conc. There were more than 5 test conc (the recommended number) used for TCE. The cell density in the test cultures amounted to 5 " 10 <sup>3</sup> cells/ml at the beginning of the assays.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Glass flasks which are recommended in OECD 201. Temp and ph were within recommended ranges.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	EC10s and EC50s were determined and reported.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control					
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Study Citation:	Brack, W., Rottler, H.. 1994. Toxicity testing of highly volatile chemicals with green algae: A new assay. 1:223-228				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	661061				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study groups in environmental conditions or other factors that would influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Authors do not report attrition for each study group or the control, but do say that the effective concentration was controlled analytically, leaving only minor uncertainties. Authors report, "EC10 and 72 <sup>h</sup> EC50, which are the effective concentration of a chemical by which algal growth is reduced by 10 percent or 50 percent compared to a control in 72 h, respectively, and the 95 percent confidence intervals of these values are shown in Table 3 (- p. 226)."
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Authors reported using the PROBIT routine of SAS.
	Metric 22: Reporting of Data	Medium	× 2	4	Figure 3 shows the results of the tests at each conc for each chemical but it's difficult to determine the exact concentrations from the figure, so some minor uncertainties remain.
	Metric 23: Explanation of Unexpected Outcomes	Medium	× 1	2	SDs were provided, but it was unclear whether or not there were any unexpected outcomes, leaving minor uncertainties.
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} \right\rceil & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

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 43:149-157					
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates					
Hero ID:	676758					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
Domain 1: Test Substance						
	Metric 1: Test Substance Identity	High	× 2	2	Test chemical was identified by name	
	Metric 2: Test Substance Source	Low	× 1	3	Source was not reported	
	Metric 3: Test Substance Purity	Low	× 1	3	The purity was not reported.	
Domain 2: Test Design						
	Metric 4: Negative Controls	Low	× 2	6	It is implied that they used a control because they mention using a "blank" to calculate values and show a growth curve for cells in the blank, but there is not much information about what is in the blank.	
	Metric 5: Negative Control Response	N/A		N/A	This is an acute study with lots of chemicals reported, and they did not report on the control response for each chemical.	
	Metric 6: Randomized Allocation	Low	× 1	3	There was no mention of random allocation.	
Domain 3: Exposure Characterization						
	Metric 7: Experimental System/Test Media Preparation	Medium	× 2	4	The system was closed for TCE which is volatile, but TCE's system was not described in detail (no info about headspace in glass, etc)	
	Metric 8: Consistency of Exposure Administration	Low	× 1	3	There were differences in how exposure was administered but because the point of the study was to figure out what housing conditions were best for this type of protozoa. These differences could have effected the EC50 reported. Authors report that some of the temperatures, and amount of food changed the growth rate of the protozoa.	
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	Measurements were not reported	
	Metric 10: Exposure Duration and Frequency	Medium	× 1	2	Various exposure durations were tested to determine which duration was most effective.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	no information was provided on number of exposure groups or spacing of exposures.	
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Study Citation: Yoshioka, Y., Ose, Y., Sato, T.. 1985. Testing for the toxicity of chemicals with *Tetrahymena pyriformis*. Science of the Total Environment 43:149-157  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 676758

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Tetrahymena pyriformis was preserved in a sterile medium of 2 percent proteasepeptone at 20° C which was renewed at 2-4 week intervals. Unsure but it sounds like they cultured their own animals in the lab from descriptions of previous studies in this paper. Acknowledgements say "Pr. Nozawa of Gifu University for providing T. pyriformis in germ-free condition"
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Number of test organisms and replicates were not reported for the test groups. It was reported that 20 cells per slide were counted using one method of counting, but that was the only number provided.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	In some of the housing conditions the animals did not do as well, but that did not significantly change the EC50 values. The point of the test was to try out different housing conditions. it looks like for testing conditions they used 24 hour test time and conditions of no 4 for culturing (Pre-culture: temp 30, hours 24; test culture: medium PRO, temp 30 type VP (cultured in vertical vessel with a porous silicone rubber stopper).
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Medium	× 2	4	They describe two different methods for counting the cells but some uncertainties remain e.g., which method they went with.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	The assessment protocol was reported with minor uncertainties.
Domain 6: Confounding / Variable Control					
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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 43:149-157				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	676758				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	"In spite of the considerable influence of the composition of the medium on the growth curve, as shown in Fig. 3 (compare No. 1 with No. 5 and No. 2 with No. 6), there was no significant difference between the EC50 value in 2 percent proteose peptone (No. 1) and that in 2 percent polypeptone (No. 5), which were both cultured at 30°C. However, the EC50 value of No. 6 differs significantly from that of No. 2, and the difference between them increased as the test period was increased from 24 to 72 h."
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	They do mention, "When the culture was contaminated by the bacterium, the test was repeated." and the effects on growth from test medium (food) was not statistically significant for the EC50 values.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	The effective concentration 50 percent (EC50) values were obtained by plotting the relative growth rates against the concentration of chemical on logarithmic probability paper.
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings were not shown for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were explained.
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: McDaniel, T., Martin, P., Ross, N., Brown, S., Lesage, S., Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109  
 Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name.
Metric 2:	Test Substance Source	High	× 1	1	Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge).
Metric 3:	Test Substance Purity	Medium	× 1	2	"Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge)." Only minor uncertainties about the purity being at 95 percent, analytical-grade.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Three replicates of embryos were also raised in uncontaminated groundwater as controls.
Metric 5:	Negative Control Response	High	× 1	1	Control mortality was reported in table 2 for each species, and deformities in controls were reported in figure 1. Control response for mortality for wood frogs, green frogs and spotted salamanders were all below 10 percent; for American toads it was about 10.1 percent with one of the replicates having a very high 26 percent mortality rate, so there are uncertainties for this species for this metric. Figure 1 shows that the negative control response for all species for percent mortality is below 10 percent. ASTM guidelines indicate "An acceptable clutch of eggs has the capability of developing into Developmental Stage 46 tadpoles with less than 10 percent gross abnormalities and less than 10 percent mortality."
Metric 6:	Randomized Allocation		× 1	0	For the acute study it was not reported whether the animals were distributed randomly.
Domain 3: Exposure Characterization					

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Study Citation: McDaniel, T.,Martin, P.,Ross, N.,Brown, S.,Lesage, S.,Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109  
Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
Hero ID: 700434

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 7: Experimental System/Test Media Preparation	High	× 2	2	Containers were covered and sealed and efforts were made to minimize head space. Authors reported that TCE solutions declined by 50 to 80 percent over the 24 hour period between renewals. Authors also mentioned, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution. The lids on the jars were sealed to reduce volatilization. Dissolved oxygen levels never fell below 80 percent saturation. Three replicates of embryos were also raised in uncontaminated groundwater as controls. Temperature was maintained at 23 ±1°C using a water bath. All tests were conducted under a 14L/10D light regime. An exhaust hood over the water bath ensured the removal of accidental gaseous PCE, TCE, and DCE volatilized from the exposure vessels."

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Study Citation: McDaniel, T., Martin, P., Ross, N., Brown, S., Lesage, S., Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109  
 Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Authors indicate that there may have been some losses of test chemical during decanting test solutions and during the placing of eggs in test chambers, and while testing a subsample at 1 hour of exposure TCE conc were only within 70 percent of nominal. The authors report, "Stock solutions of TCE, PCE, and DCE were dissolved in local groundwater in a dilution series. Groundwater was used as the medium for acute tests to emulate conditions in surface waters fed by chloroethylene- contaminated groundwater. Nominal test concentrations were as follows: PCE-2.5, 7.5, 12.5, and 20 mg/L; TCE-12.5, 20, 40, and 60 mg/L; and cis- and trans-DCE-12.5, 60, and 100 mg/L. Based on the results of initial exposures of American toad embryos, a second exposure was conducted with elevated concentrations of PCE and TCE as follows: PCE-15, 30, and 45 mg/L; and TCE-35, 55, and 85 mg/L. Maximum exposure concentrations of PCE and TCE were limited by the compounds' solubility in groundwater. Concentrations of test solutions, including controls, were measured at 24 h (just prior to solution renewal, see below). Concentrations at t = 0 h were based on dilutions of measured stock solutions. Some losses occurred while decanting test solutions and during the placing of eggs in test chambers. In a subsample of test solutions measured at 1 h of exposure, concentrations of PCE were within 99 percent of nominal, while cis- and trans-DCE were within 90 percent. However, levels of TCE were only within 70 percent of nominal."

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 Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	Measured concentrations were taken, and used to derive an EC50. . Authors reported "To prevent volatilization, a glass flask (6 L) was filled to capacity with stock solution to reduce head space and then stirred using a stir bar at low speed to prevent disruption of the surface. Stock solutions were stirred until the compound was dissolved or until the solution had reached saturation (between 2 and 48 h, depending on the compound). Despite these precautions, actual concentrations tended to be less than nominal concentrations. For this reason, concentrations of stock solutions were measured prior to mixing of the test solutions, using Purge and Trap GC/MS according to U.S. EPA method 82 60B (U.S. EPA 1996), to ensure that test solutions were within 10 percent of the nominal concentration at 1h of exposure. The GS/MS was performed on a Hewlett Packard 6890 gas chromatograph, using a Hewlett Packard 5973 mass selective detector, a 3100 purge and trap concentrator, and a PTA-30 purge and trap autosampler. The method detection limit for all chloroethylene analyses was 1 ppb.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	ASTM guidelines for FETAX on American clawed frog suggest 96 hours and a static renewal set up, renewed every 24 hours, which this study does for the acute test.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	This study had four exposure groups for TCE and ASTM FETAX Guidelines suggests the following "At a minimum, five concentrations for each endpoint are used. However, additional concentrations between the EC16 and EC84 are highly recommended to ensure obtaining accurate 96-hour LC50 and EC50 values."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Solubility was taken into consideration, and exposure concentrations kept below the solubility of TCE in groundwater.

Domain 4: Test Organism

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 Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 13:	Test Organism Characteristics	Medium	× 2	4	Wood frog and green frog: Test organisms seem to be sufficiently sensitive to the exposures administered to derive an EC50, but are not a suggested species in the ASTM guideline or OECD and EPA guidelines for amphibian growth and development, which suggest African clawed frog. This study instead used these species to test sensitivity for North American species. Only minor uncertainties because they are not suggested species from a guideline.
Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Study authors did not report whether animals were acclimatized or whether pretreatment conditions were the the same for treatment and controls. They authors do report, " In 2001 and 2002, egg masses of wood frogs, spotted salamanders," American toads, and green frogs were collected from a wetland not contaminated with chloroethylenes in F1amborough Township (Ontario,Canada). Water from wetlands were tested for chloroethylenes in 2001 from each site where eggs were collected. No chloroethylenes were detected; the minimum detection limit for this analysis was 1 ppb. Egg masses were less than 24 h old when exposures were initiated. For each species, three egg masses were used (with the exception of the second exposure of American toads, where only one egg mass was used). Each egg.mass was from a different female and represented a replicate. Thus, there were three replicate jars for each chemical by concentration combination, for a total of 45 jars per species. Eggs were not dejellied prior to exposure to more accurately imitatenatural exposure conditions. Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution."
Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	ASTM guidelines suggest 20-25 and two replicates, and the study authors reported, "there were three replicate jars for each chemical" and, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution."

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 Data Type: Other; Aquatic; other amphibian - wood frog and green frog  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Animals were held in 1 L glass mason jars containing 300 ml of test solution. Jars were sealed and temperature was maintained at 23±1 degree C using a water bath. All tests were conducted under 14L/10D light regime. The ASTM guidelines recommend glass, and this temperature is appropriate for african clawed frog but unsure if this temperature is also appropriate for these north american species. Additionally the photoperiod is longer than the one recommended in the ASTM Guidelines.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. LC50 and EC50 were reported which are the recommended outcomes in ASTM guidelines for FETAX.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	All animals were assessed at the end of the 96 hour period with minor uncertainties due to incomplete reporting.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Controls for the wood frogs and green frogs were under 10 percent mortality and deformities. Details on attrition unrelated to exposure for each exposure concentration were also reported as the average with a range. There is a wide range of portailities between the replicates.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	A two-factor ANOVA was used. ASTM FETAX Guidelines suggests either probit analysis, trimmed Spearman-Karber analysis, or the two-point graphical method to estimate LC50 and EC50 values.

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Data Type:	Other; Aquatic; other amphibian - wood frog and green frog				
Hero ID:	700434				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 22: Reporting of Data	Medium	× 2	4	Data was reported for each exposure group in either table or graphical form. It's hard to tell the exact numbers from the graphical representation of the EC50 values for each exposure level, resulting in minor uncertainties.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were explained. (e.g., loss of TCE through evaporation, damaged eggs in transit)
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

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 Data Type: Other; Aquatic; other amphibian - american toad  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name.
Metric 2:	Test Substance Source	High	× 1	1	Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge).
Metric 3:	Test Substance Purity	Medium	× 1	2	"Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge)." Only minor uncertainties about the purity being at 95 percent, analytical-grade.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Three replicates of embryos were also raised in uncontaminated groundwater as controls.
Metric 5:	Negative Control Response	Medium	× 1	2	Control mortality was reported in table 2 for each species, and deformities in controls were reported in figure 1. Control response for mortality for wood frogs, green frogs and spotted salamanders were all below 10 percent; for American toads it was about 10.1 percent with one of the replicates having a very high 26 percent mortality rate, so there are uncertainties for this species for this metric. Authors threw those numbers out and indicated that the high mortality rate for that replicate could have been due to damage the eggs recieved in transit. Figure 1 shows that the negative control response for all species for percent mortality is below 10 percent. ASTM guidelines indicate "An acceptable clutch of eggs has the capability of developing into Developmental Stage 46 tadpoles with less than 10 percent gross abnormalities and less than 10 percent mortality."
Metric 6:	Randomized Allocation	Low	× 1	3	For the acute study it was not reported whether the animals were distributed randomly.

Domain 3: Exposure Characterization

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Study Citation: McDaniel, T.,Martin, P.,Ross, N.,Brown, S.,Lesage, S.,Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109  
Data Type: Other; Aquatic; other amphibian - american toad  
Hero ID: 700434

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Containers were covered and sealed and efforts were made to minimize head space. Authors reported that TCE solutions declined by 50 to 80 percent over the 24 hour period between renewals. Authors also mentioned, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution. The lids on the jars were sealed to reduce volatilization. Dissolved oxygen levels never fell below 80 percent saturation. Three replicates of embryos were also raised in uncontaminated groundwater as controls. Temperature was maintained at 23 +/-1°C using a water bath. All tests were conducted under a 14L/10D light regime. An exhaust hood over the water bath ensured the removal of accidental gaseous PCE, TCE, and DCE volatilized from the exposure vessels."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Authors indicate that there may have been some losses of test chemical during decanting test solutions and during the placing of eggs in test chambers, and while testing a subsample at 1 hour of exposure TCE conc were only within 70 percent of nominal. The authors report, "Stock solutions of TCE, PCE, and DCE were dissolved in local groundwater in a dilution series. Groundwater was used as the medium for acute tests to emulate conditions in surface waters fed by chloroethylene- contaminated groundwater. Nominal test concentrations were as follows: PCE-2.5, 7.5, 12.5, and 20 mg/L; TCE-12.5, 20, 40, and 60 mg/L; and cis- and trans-DCE-12.5, 60, and 100 mg/L. Based on the results of initial exposures of American toad embryos, a second exposure was conducted with elevated concentrations of PCE and TCE as follows: PCE-15, 30, and 45 mg/L; and TCE-35, 55, and 85 mg/L. Maximum exposure concentrations of PCE and TCE were limited by the compounds' solubility in groundwater. Concentrations of test solutions, including controls, were measured at 24 h (just prior to solution renewal, see below). Concentrations at t = 0 h were based on dilutions of measured stock solutions. Some losses occurred while decanting test solutions and during the placing of eggs in test chambers. In a subsample of test solutions measured at 1 h of exposure, concentrations of PCE were within 99 percent of nominal, while cis- and trans-DCE were within 90 percent. However, levels of TCE were only within 70 percent of nominal."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	Measured concentrations were taken, and used to derive an EC50. . Authors reported "To prevent volatilization, a glass flask (6 L) was filled to capacity with stock solution to reduce head space and then stirred using a stir bar at low speed to prevent disruption of the surface. Stock solutions were stirred until the compound was dissolved or until the solution had reached saturation (between 2 and 48 h, depending on the compound). Despite these precautions, actual concentrations tended to be less than nominal concentrations. For this reason, concentrations of stock solutions were measured prior to mixing of the test solutions, using Purge and Trap GC/MS according to U.S. EPA method 82 60B (U.S. EPA 1996), to ensure that test solutions were within 10 percent of the nominal concentration at 1h of exposure. The GS/MS was performed on a Hewlett Packard 6890 gas chromatograph, using a Hewlett Packard 5973 mass selective detector, a 3100 purge and trap concentrator, and a PTA-30 purge and trap autosampler. The method detection limit for all chloroethylene analyses was 1 ppb.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	ASTM guidelines for FETAX on American clawed frog suggest 96 hours and a static renewal set up, renewed every 24 hours, which this study does for the acute test.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	This study had four exposure groups for TCE and ASTM FETAX Guidelines suggests the following "At a minimum, five concentrations for each endpoint are used. However, additional concentrations between the EC16 and EC84 are highly recommended to ensure obtaining accurate 96-hour LC50 and EC50 values." For American toads the concentrations were too low to generate either an LC50 or an EC50.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Solubility was taken into consideration, and exposure concentrations kept below the solubility of TCE in groundwater.

Domain 4: Test Organism

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 13:	Test Organism Characteristics	Medium	× 2	4	American toads: Test organisms seem to be sufficiently sensitive to the exposures administered to derive an EC50, but are not a suggested species in the ASTM guideline or OECD and EPA guidelines for amphibian growth and development which suggest African clawed frog. This study instead used these species to test sensitivity for North American species. Only minor uncertainties because they are not suggested species from a guideline.
Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Study authors did not report whether animals were acclimatized or whether pretreatment conditions were the the same for treatment and controls. They authors do report, " In 2001 and 2002, egg masses of wood frogs, spotted salamanders," American toads, and green frogs were collected from a wetland not contaminated with chloroethylenes in F1amborough Township (Ontario,Canada). Water from wetlands were tested for chloroethylenes in 2001 from each site where eggs were collected. No chloroethylenes were detected; the minimum detection limit for this analysis was 1 ppb. Egg masses were less than 24 h old when exposures were initiated. For each species, three egg masses were used (with the exception of the second exposure of American toads, where only one egg mass was used). Each egg.mass was from a different female and represented a replicate. Thus, there were three replicate jars for each chemical by concentration combination, for a total of 45 jars per species. Eggs were not dejellied prior to exposure to more accurately imitatenatural exposure conditions. Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution."
Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	ASTM guidelines suggest 20-25 and two replicates, and the study authors reported, "there were three replicate jars for each chemical" and, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Animals were held in 1 L glass mason jars containing 300 ml of test solution. Jars were sealed and temperature was maintained at 23±1 degree C using a water bath. All tests were conducted under 14L/10D light regime. The ASTM guidelines recommend glass, and this temperature is appropriate for african clawed frog but unsure if this temperature is also appropriate for these north american species. Additionally the photoperiod is longer than the one recommended in the ASTM Guidelines.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. LC50 and EC50 were reported which are the recommended outcomes in ASTM guidelines for FETAX.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	All animals were assessed at the end of the 96 hour period with minor uncertainties due to incomplete reporting.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	One of the controls for the American toads had very high mortality 26 percent, results from that clutch were removed. authors suggest the egg mass may have been damaged in transit. Details on attrition unrelated to exposure for each exposure concentration were also reported as the average with a range. There is a wide range of mortalities between the replicates, and zero mortality at the highest concentration.
Domain 7: Data Presentation and Analysis					

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Data Type:	Other; Aquatic; other amphibian - american toad				
Hero ID:	700434				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 21: Statistical Methods	High	× 1	1	A two-factor ANOVA was used. ASTM FETAX Guidelines suggests either probit analysis, trimmed Spearman-Karber analysis, or the two-point graphical method to estimate LC50 and EC50 values.
	Metric 22: Reporting of Data	Medium	× 2	4	Data was reported for each exposure group in either table or graphical form. It's hard to tell the exact numbers from the graphical representation of the EC50 values for each exposure level, resulting in minor uncertainties.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were explained. (e.g., loss of TCE through evaporation, damaged eggs in transit)
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: McDaniel, T., Martin, P., Ross, N., Brown, S., Lesage, S., Pauli, B.. 2004. Effects of chlorinated solvents on four species of North American amphibians. Archives of Environmental Contamination and Toxicology 47:101-109  
 Data Type: Other; Aquatic; other amphibian - spotted salamder  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Chemical was identified by name.
	Metric 2: Test Substance Source	High	× 1	1	Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge).
	Metric 3: Test Substance Purity	Medium	× 1	2	"Stock solutions were made from 95 percent pure, analytical-grade PCE, TCE, and cis- and trans-DCE (Sigma-Aldridge)." Only minor uncertainties about the purity being at 95 percent, analytical-grade.
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	Three replicates of embryos were also raised in uncontaminated groundwater as controls.
	Metric 5: Negative Control Response	High	× 1	1	Control mortality was reported in table 2 for each species, and deformities in controls were reported in figure 1. Control response for mortality for wood frogs, green frogs and spotted salamanders were all below 10 percent; for American toads it was about 10.1 percent with one of the replicates having a very high 26 percent mortality rate, so there are uncertainties for this species for this metric. Figure 1 shows that the negative control response for all species for percent mortality is below 10 percent. ASTM guidelines indicate "An acceptable clutch of eggs has the capability of developing into Developmental Stage 46 tadpoles with less than 10 percent gross abnormalities and less than 10 percent mortality."
	Metric 6: Randomized Allocation	Low	× 1	3	For the acute study it was not reported whether the animals were distributed randomly.
Domain 3: Exposure Characterization					

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 Data Type: Other; Aquatic; other amphibian - spotted salamander  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 7: Experimental System/Test Media Preparation	Low	× 2	6	Containers were covered and sealed but there was no mention of minimizing head space, and authors mentioned that TCE solutions declined by 50 to 80 percent over the 24 hour period between renewals. Authors also mentioned, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution. The lids on the jars were sealed to reduce volatilization. Dissolved oxygen levels never fell below 80 percent saturation. Three replicates of embryos were also raised in uncontaminated groundwater as controls. Temperature was maintained at 23 ±1°C using a water bath. All tests were conducted under a 14L/10D light regime. An exhaust hood over the water bath ensured the removal of accidental gaseous PCE, TCE, and DCE volatilized from the exposure vessels."

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 Data Type: Other; Aquatic; other amphibian - spotted salamander  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Authors indicate that there may have been some losses of test chemical during decanting test solutions and during the placing of eggs in test chambers, and while testing a subsample at 1 hour of exposure TCE conc were only within 70 percent of nominal. The authors report, "Stock solutions of TCE, PCE, and DCE were dissolved in local groundwater in a dilution series. Groundwater was used as the medium for acute tests to emulate conditions in surface waters fed by chloroethylene- contaminated groundwater. Nominal test concentrations were as follows: PCE-2.5, 7.5, 12.5, and 20 mg/L; TCE-12.5, 20, 40, and 60 mg/L; and cis- and trans-DCE-12.5, 60, and 100 mg/L. Based on the results of initial exposures of American toad embryos, a second exposure was conducted with elevated concentrations of PCE and TCE as follows: PCE-15, 30, and 45 mg/L; and TCE-35, 55, and 85 mg/L. Maximum exposure concentrations of PCE and TCE were limited by the compounds' solubility in groundwater. Concentrations of test solutions, including controls, were measured at 24 h (just prior to solution renewal, see below). Concentrations at t = 0 h were based on dilutions of measured stock solutions. Some losses occurred while decanting test solutions and during the placing of eggs in test chambers. In a subsample of test solutions measured at 1 h of exposure, concentrations of PCE were within 99 percent of nominal, while cis- and trans-DCE were within 90 percent. However, levels of TCE were only within 70 percent of nominal."

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 Data Type: Other; Aquatic; other amphibian - spotted salamander  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Measured concentrations were taken, and used to derive an EC50. Authors reported "To prevent volatilization, a glass flask (6 L) was filled to capacity with stock solution to reduce head space and then stirred using a stir bar at low speed to prevent disruption of the surface. Stock solutions were stirred until the compound was dissolved or until the solution had reached saturation (between 2 and 48 h, depending on the compound). Despite these precautions,actual concentrations tended to be less than nominal concentrations.For this reason, concentrations of stock solutions were measured prior to mixing of the test solutions, using Purge and Trap GC/MS according to U.S. EPA method 82 60B (U.S. EPA 1996), to ensure that test solutions were within 10 percent of the nominal concentration at 1h of exposure. The GS/MS was performed on a Hewlett Packard 6890 gas chromatograph, using a Hewlett Packard 5973 mass selective detector, a 3100 purge and trap concentrator, and a PTA-30 purge and trap autosampler. The method detection limit for all chloroethylene analyses was 1 ppb.

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 Data Type: Other; Aquatic; other amphibian - spotted salamander  
 Hero ID: 700434

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	ASTM guidelines for FETAX on American clawed frog suggest 96 hours and a static renewal set up, renewed every 24 hours, which this study does for the acute test. However, it appears that 96 hours is not enough time for salamanders. Authors state, "Exposures followed a 96-h static renewal process with test solutions refreshed daily. Most eggs hatched during the 96-h exposure period with the exception of spotted sal anders. After 96 h, survivorship was assessed; larvae were then euthanized with a solution of clove oil. Spotted salamanders had not hatched by the end of the 96-h period since they take up to a week longer to develop to hatching than the anuran species chosen. The developing salamander embryos were placed in clean groundwater until hatching was complete. Anuran embryos were staged at 96 h according to Gosner (1960) to test for effects of exposures on developmental rates. Salamander larvae were staged at 192 h according to Harrison (1969). Larvae were examined for developmental deformities according to the Atlas of Abnonnalities (Bantle et al. 1998) for Xenopus laevis tadpoles.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	This study had four exposure groups for TCE and ASTM FETAX Guidelines suggests the following "At a minimum, five concentrations for each endpoint are used. However, additional concentrations between the EC16 and EC84 are highly recommended to ensure obtaining accurate 96-hour LC50 and EC50 values."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Solubility was taken into consideration, and exposure concentrations kept below the solubility of TCE in growndwater.

Domain 4: Test Organism

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 Data Type: Other; Aquatic; other amphibian - spotted salamander  
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Spotted salamander: Test organisms seem to be sufficiently sensitive to the exposures administered to derive an EC50, but are not a suggested species in the ASTM guideline or OECD and EPA guidelines for amphibian growth and development which suggest African clawed frog. This study instead used these species to test sensitivity for North American species. Only minor uncertainties because they are not suggested species from a guideline.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Study authors did not report whether animals were acclimatized or whether pretreatment conditions were the the same for treatment and controls. They authors do report, " In 2001 and 2002, egg masses of wood frogs, spotted salamanders," American toads, and green frogs were collected from a wetland not contaminated with chloroethylenes in F1amborough Township (Ontario,Canada). Water from wetlands were tested for chloroethylenes in 2001 from each site where eggs were collected. No chloroethylenes were detected; the minimum detection limit for this analysis was 1 ppb. Egg masses were less than 24 h old when exposures were initiated. For each species, three egg masses were used (with the exception of the second exposure of American toads, where only one egg mass was used). Each egg.mass was from a different female and represented a replicate. Thus, there were three replicate jars for each chemical by concentration combination, for a total of 45 jars per species. Eggs were not dejellied prior to exposure to more accurately imitatenatural exposure conditions. Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	ASTM guidelines suggest 20-25 and two replicates, and the study authors reported, "there were three replicate jars for each chemical" and, "Each egg mass was gently divided into clusters of approximately 30 eggs (with the exception of spotted salamanders with 5 to 10 eggs) and placed in a 1-L glass Mason jar containing 300 ml of test solution." This resulted in the nominal and measured conc for TCE not having an adequate sample size to generate confidence intervals. Because salamanders are difficult to rear in the lab in high numbers, this was taken into consideration in the scoring for this metric (given a low instead of an unacceptable). Additionally the number of organisms suggested in the ASTM guidelines are based on another species.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Animals were held in 1 L glass mason jars containing 300 ml of test solution. Jars were sealed and temperature was maintained at 23±1 degree C using a water bath. All tests were conducted under 14L/10D light regime. The ASTM guidelines recommend glass, and this temperature is appropriate for african clawed frog but unsure if this temperature is also appropriate for these north american species. Additionally the photoperiod is longer than the one recommended in the ASTM Guidelines.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed the intended outcomes of interest and was sensitive for the outcomes of interest. LC50 and EC50 were reported which are the recommended outcomes in ASTM guidelines for FETAX. Dead individuals were counted and removed at the end of each 24 hours.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	All animals were assessed at the end of the 96 hour period with minor uncertainties due to incomplete reporting.
Domain 6: Confounding / Variable Control					
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Hero ID:	700434				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Controls for the spotted salamanders were under 10 percent mortality and deformities. Details on attrition unrelated to exposure for each exposure concentration were also reported as the average with a range. There is a wide range of portailities between the replicates.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	A two-factor ANOVA was used. ASTM FETAX Guidelines suggests either probit analysis, trimmed Spearman-Karber analysis, or the two-point graphical method to estimate LC50 and EC50 values. However due to sample size authors were not able to generate confidence intervals.
	Metric 22: Reporting of Data	Medium	× 2	4	Data was reported for each exposure group in either table or graphical form. It's hard to tell the exact numbers from the graphical representation of the EC50 values for each exposure level, resulting in minor uncertainities.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were explained. (e.g., loss of TCE through evaporation, damaged eggs in transit)
Overall Quality Determination <sup>‡</sup>		Medium		1.8	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Fort, D.,Rayburn, J.,Deyoung, D.,Bantle, J.. 1991. Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug and Chemical Toxicology 14:143-160  
 Data Type: Other; Aquatic; other amphibian - frog  
 Hero ID: 701994

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Identified by name trichloroethylene
Metric 2:	Test Substance Source	High	× 1	1	Aldrich, Milwaukee, WI
Metric 3:	Test Substance Purity	Low	× 1	3	Not reported
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Four separate dishes of 20 embryos were exposed to FETAX solution alone and designated FETAX solution controls. Controls including FETAX solution,each MAS , CO-MAS + toxicant (negative control), cyclophosphamide (FETAX reference Aroclor 1254 MAS proteratogen7 " 9 - 11) and acetylhydrazide (additional mixed MAS positive control7), and unactivated toxicant were tested concurrently with each experiment .
Metric 5:	Negative Control Response	High	× 1	1	In this study, FETAX solution control embryo mortality and malformation rates were <2 . 0 percent and <5 percent, respectively . Mortality and malformation rates for each MAS control were <4 percent and <9 percent, respectively. Acceptable rates of FETAX solution a nd MAS control mortality and malformation are generally 1 0 percent . No less than 86 percent of those embryos subjected to 4.0 mg/ml activated cyclophosphamide and e ither the Aroclor 1254 or the mixed MAS died . Survivors of the previous treatments were severely deformed . Exposure of embryos to 3 . 0 mg/ml activated acetylhydrazide and the INH MAS or mixed MAS produced mortality and malformation rates >43 percent and 100 percent, respectively .
Metric 6:	Randomized Allocation	Low	× 1	3	Not reported how allocation was conducted
Domain 3: Exposure Characterization					
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 Data Type: Other; Aquatic; other amphibian - frog  
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Covered petri dishes were used but there was no mention of headspace or other considerations for volatility.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Only general details of exposure administration were given. All solutions were renewed every 24 hours in a static renewal system for 96 hours.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Not reported how test concentrations were determined, and because substance is volatile could have substantial impacts on results.

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Study Citation: Fort, D.,Rayburn, J.,Deyoung, D.,Bantle, J.. 1991. Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug and Chemical Toxicology 14:143-160  
 Data Type: Other; Aquatic; other amphibian - frog  
 Hero ID: 701994

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 10:	Exposure Duration and Frequency	High	× 1	1	Published protocol for FETAX suggest a static-renewal, 96 hour test, which is what the authors of this paper conducted in this study. While it's not an OECD or EPA guideline study, other peer reviewed publications have used FETAX repeatedly, and the ASTM guidelines methods were reviewed by NTP at the request of EPA, and determined that FETAX data can be used to identify and prioritize sites with increased developmental toxicity risks. "Due to varying susceptibilities among animals, testing in multiple species is considered necessary to protect the environment. For each species, it is a combination of toxicants, water quality, and the organism itself that defines the hazard for a specific concentration of a toxicant within defined water quality conditions. Ecotoxicological standards are generally based on the susceptibility of the adult animal, which may not provide adequate protection for embryonic development and reproduction in many species. It is inherently impossible to evaluate developmental toxicity without exposing animals throughout development and assessing for adverse effects in multiple life stages. and for Early embryonic and juvenile stages are often the most susceptible periods for the toxic effects of many environmental contaminants. Embryonic development in amphibians is sensitive to water quality. Because ofthis, FETAX has been used in ecotoxicological studies to evaluate the potential developmental hazard of contaminated surface waters, sediments, waste site soils, and industrial wastewater and to evaluate the efficacy of wastewater treatment procedures. In this context, the resulting data can be used to identify and prioritize sites with increased developmental toxicity risks. "
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	For each compound, 12-18 concentrations were tested with replicates. FETAX publications suggest at least 5 test conc are appropriate. Also the study reports "For each compound, one range-finding and two definitive dose-response assays were conducted with and without the MAS."

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 Data Type: Other; Aquatic; other amphibian - frog  
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Xenopus laevis was used in this study and is the recommended species in ASTM guidelines.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Uncertainties remain because the acclimatization was not fully described in this paper. Authors write, "Xenopus adult care, breeding, and embryo collection were performed as described by Dawson and Bantle."
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	20 embryos were used in replicate. Guidelines for ASTM FETAX state "Each of the three definitive tests is conducted using embryos from a different male/female pair of X. laevis. Each test consists of several different concentrations of the test substance with two replicate dishes at each test concentration and four replicate dishes for each control. Each plastic or glass Petri dish contains 20 or 25 embryos, respectively. The number of embryos per dish, the number of replicate dishes per test substance concentration, and the number of replicate tests per study were not based on a formal scientific analysis. Rather, selection was based on the best scientific judgement of the developers/users of the assay at the time the ASTM FETAX Guideline (1991, 1998) was prepared (J. Bantle and D. Fort, personal communication)."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Minor uncertainties about housing. Temp and ph are appropriate according to published recommendations. "For experiments conducted without the MAS, groups of 20 embryos were placed in 60 mm covered plastic Petri dishes with varying concentrations of the appropriate test compound." "Each treatment dish contained a total o f 8 ml o f solution." "The pH of all stock solutions was 7.0. Embryos were cultured at 23° 1" c. All solutions were renewed every 24 h. Dead embryos were removed at this time ." The ASTM guidance states "Glass, Type 316 stainless steel, nylon, and fluorocarbon plastic should be used whenever possible to minimize dissolution, leaching, and sorption." It is unclear what kind of plastic these petri dishes are made of.
Domain 5: Outcome Assessment	Metric 17: Outcome Assessment Methodology	High	× 2	2	ASTM guidance states that "Only those biological responses related to mortality and malformations are considered in this analysis" and an LC50 and EC50 for malformations were established.The study states, "96-h median lethal concentrations (LC50) and the concentrations inducing gross terata in 50 percent of the surviving larvae (EC50) along with respective 95 percent confidence limits were determined using Litchfield Wilcoxon probit analysis . 20 A Teratogenic Index (TI=LC50/EC50) was used as a meth6d of assessing teratogenic potential . 5 18- ll, 21 Head-tail length of surviving embryos was measured as an index of embryo growth. Minimum concentrations to inhibit growth (MCIG) were determined with t he t-Test (p<0.05)."
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol
Domain 6: Confounding / Variable Control	Metric 19: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Incomplete data across study groups, and there could be confounding due to the volatility of the chemical.

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Study Citation: Fort, D.,Rayburn, J.,Deyoung, D.,Bantle, J.. 1991. Assessing the efficacy of an Aroclor 1254-induced exogenous metabolic activation system for FETAX. Drug and Chemical Toxicology 14:143-160  
 Data Type: Other; Aquatic; other amphibian - frog  
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	There was some discussion of health outcomes unrelated to exposure with only minor uncertainties about why there were differences between the various controls. "In this study, FETAX solution control embryo mortality and malformation rates were <2.0 percent and <5 percent, respectively. Mortality and malformation rates for each MAS control were <4 percent and <9 percent, respectively. Acceptable rates of FETAX solution and MAS control mortality and malformation are generally 10 percent. No less than 86 percent of those embryos subjected to 4.0 mg/ml activated cyclophosphamide and either the Aroclor 1254 or the mixed MAS died. Survivors of the previous treatments were severely deformed. Exposure of embryos to 3.0 mg/ml activated acetylhydrazide and the INH MAS or mixed MAS produced mortality and malformation rates >43 percent and 100 percent, respectively."
Domain 7: Data Presentation and Analysis	Metric 21: Statistical Methods	High	× 1	1	ASTM guidance says that either probit analysis, trimmed Spearman-Kärber analysis or the two-point graphical methods are used to estimate the LC50 and EC50 values. This study states that the "96-h median lethal concentrations (LC50) and the concentrations inducing gross terata in 50 percent of the surviving larvae (EC50) along with respective 95 percent confidence limits were determined using Litchfield Wilcoxon probit analysis. A Teratogenic Index (TI=LC50/EC50) was used as a method of assessing teratogenic potential. Head-tail length of surviving embryos was measured as an index of embryo growth. Minimum concentrations to inhibit growth (MCIG) were determined with the t-Test (p<0.05)."
	Metric 22: Reporting of Data	Medium	× 2	4	Some information is given for each treatment group, but it's incomplete. information was given for TCE alone, TCE and MAS, etc but not for each exposure group

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Data Type:	Other; Aquatic; other amphibian - frog				
Hero ID:	701994				

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were discussed. "Several of the TCE-induced malformations including cardiac effects observed in the avian modelare similar to those recorded with Xenopus. Differences in the ability of TCE to induce terata in mammals, birds, and frog embryos may be the result of differences in embryonic susceptibility across the phylogenetic range, route of exposure, as well as, pharmacological nature of the test system. Themajority of mammalian studies utilized pulmonary exposure, whereas, the avian system and FETAX provide direct embryonic exposure to toxicants. Positive results in FETAX may, in fact, be due to highly embryotoxic intermediates or metabolites of TCE. The formation of a reactive epoxide intermediate (TCEoxide) has been identified in rat, rabbit, and mouse."
Overall Quality Determination <sup>‡</sup>		Medium		1.8	
Extracted		Yes			

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Fort, D.,Rogers, R.,Stover, E.,Finch, R.. 2001. Optimization of an exogenous metabolic activation system for FETAX. I. Post-isolation rat liver microsome mixtures. Drug and Chemical Toxicology 24:103-115  
 Data Type: Other; Aquatic; other amphibian - frog  
 Hero ID: 701995

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name
Metric 2:	Test Substance Source	High	× 1	1	Sigma St Louise Missouri
Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Four separate dishes of 20 embryos were exposed FETAX solution alone and designated FETAX solution controls.
Metric 5:	Negative Control Response	High	× 1	1	In this study, FETAX solution control embryo mortality and malformation rates were 5.0 percent and 6.3 percent, respectively. Mortality and malformation rates for each MAS control were 7.5 percent and 7.6 percent, respectively.
Metric 6:	Randomized Allocation	Low	× 1	3	It was not reported how animals were allocated
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Dishes were covered but no mention of headspace minimizing was given. TCE is very volatile, and it was not indicated whether concentrations were confirmed through measurement.
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Only general details of exposure administration were given. This study reports that solutions were renewed every 24 hours in a static renewal system for 96 hours, and "For each compound, 12"18 concentrations were tested in duplicate. Four separate dishes of 20 embryos were exposed FETAX solution alone and designated FETAX solution controls. Each treatment dish contained a total of 10mL of solution."

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether test concentrations were measured, and based on professional judgment of experiment design and nature of test substance, actual concentration cannot be expected to be similar to nominal concentration.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	FETAX is a static-renewal bioassay, and in accordance with other published FETAX assays (and the ASTM guidelines which were reviewed by NTP), the study authors did a 96 hour test with the solutions renewed every 24 hours.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	"For each compound, one range-finding and two definitive dose-responseassays were conducted with and without the MASs." and "For each compound,12"18 concentrations were tested in duplicate." FETAX publications suggest at least 5 test conc are appropriate.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Xenopus laevis are used in this study are the recommended species for FETAX in ASTM guidelines
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	The study reports, "X. laevis adult care, breeding, and embryo collection were preformed as described in ASTM E1439-98" and it is not clear from this paper whether animals were acclimatized.

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Study Citation: Fort, D.,Rogers, R.,Stover, E.,Finch, R.. 2001. Optimization of an exogenous metabolic activation system for FETAX. I. Post-isolation rat liver microsome mixtures. Drug and Chemical Toxicology 24:103-115  
 Data Type: Other; Aquatic; other amphibian - frog  
 Hero ID: 701995

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	This study used 20 in replicate in plastic dishes. Guidelines for ASTM FETAX state "Each of the three definitive tests is conducted using embryos from a different male/female pair of <i>X. laevis</i> . Each test consists of several different concentrations of the test substance with two replicate dishes at each test concentration and four replicate dishes for each control. Each plastic or glass Petri dish contains 20 or 25 embryos, respectively. The number of embryos per dish, the number of replicate dishes per test substance concentration, and the number of replicate tests per study were not based on a formal scientific analysis. Rather, selection was based on the best scientific judgement of the developers/users of the assay at the time the ASTM FETAX Guideline (1991, 1998) was prepared (J. Bantle and D. Fort, personal communication)."
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Minor uncertainties about housing. The ASTM guidance on FETAX suggest 23 degrees C +-3 which was maintained in this study, and ph was maintained between the suggested 6.5 and 9.0 range with 7.7 being optimal. Each treatment dish contained a total of 10mL of solution for 20 embryos each which is enough according to ASTM Guidance. This guidance also states "Glass, Type 316 stainless steel, nylon, and fluorocarbon plastic should be used whenever possible to minimize dissolution, leaching, and sorption." It is unclear what kind of plastic these petri dishes are made of.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology addressed the intended outcomes of interest. ASTM guidance states that "Only those biological responses related to mortality and malformations are considered in this analysis" and an LC50 and EC50 for malformations were established.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol

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Data Type:	Other; Aquatic; other amphibian - frog				
Hero ID:	701995				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
Metric 19:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Incomplete data across study groups, and there could be confounding due to the volatility of the chemical.
Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	The study reports "In this study, FETAX solution control embryo mortality and malformation rates were 5.0 percent and 6.3 percent, respectively. Mortality and malformation rates for each MAS control were 7.5 percent and 7.6 percent, respectively. Acceptable rates of FETAX solution and MAS control mortality and malformation are generally 10 percent."
Domain 7: Data Presentation and Analysis					
Metric 21:	Statistical Methods	High	× 1	1	ASTM guidance says that either probit analysis, trimmed Spearman-Karber analysis or the two-point graphical methods are used to estimate the LC50 and EC50 values. The study reports, "96 h median lethal concentrations (LC50) and the concentrations inducing gross terata in 50 percent of the surviving larvae (EC50) along with respective 95 percent fiducial limits were determined using trimmed Spearman-Karber analysis. Head-tail length of surviving embryos was measured as an index of embryo growth. Minimum concentrations to inhibit growth (MCIG) were determined using ANOVA [Bonferroni t-test (p<0.05)]."
Metric 22:	Reporting of Data	Low	× 2	6	Data for exposure related findings were not shown for each study group but results were described in the text.
Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes.
Overall Quality Determination <sup>‡</sup>		Medium		1.8	
Extracted		Yes			
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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. *Ecotoxicology and Environmental Safety* 39:136-146  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE identified by name
Metric 2:	Test Substance Source	High	× 1	1	Test compounds (Table 1) were obtained from Aldrich Chemical (Milwaukee, Wisconsin). All were widely employed industrial chemicals and have a mode-of-action classified as nonpolar narcosis (Schultz et al., 1990; Van Leeuwen et al., 1992; Russon et al., 1997).
Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity is reported as 99.5 percent as labeled but not independently verified.
Domain 2: Test Design					
Metric 4:	Negative Controls	Low	× 2	6	Authors reported using negative controls but did not report details of the negative control group.
Metric 5:	Negative Control Response	High	× 1	1	Negative control response for mortality for 2-day study reported in table 4.
Metric 6:	Randomized Allocation	Low	× 1	3	Not randomly allocated
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The test was conducted in a closed container and filled to capacity to limit volatilization. "25-ml borosilicate glass vials filled to capacity and closed tightly using teflon PTFE-lined silicon septa held in place by polypropylene screw-on caps. These vials are sold by scientific suppliers as 'EPA vials' for storage of water samples. Masten et al. (1994) found that static-renewal tests with these vials maintained concentrations of volatile chemicals more successfully than flowthrough test designs."
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Only minor uncertainties about exposure administration

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 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	"In tests with individual components, toxicant concentrations were measured at the beginning and end of the experiment. Newly prepared test solution and 24-h-old test solution composited from three replicates from each treatment level were analyzed. Acute toxicological results are based on the geometric mean of the two measured concentrations for each treatment level measured at the beginning of the test."
	Metric 10: Exposure Duration and Frequency	High	× 1	1	48 hour test for daphnia
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	EPA effluent guidelines for C. dubia recommends 5 test concentrations and there were 5 in this acute test
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Ceriodaphnia dubia is suitable and recommended in EPA effluent test guidelines. <a href="https://www.epa.gov/sites/production/files/2015-12/documents/method_1002_2002.pdf">https://www.epa.gov/sites/production/files/2015-12/documents/method_1002_2002.pdf</a>
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	The study does not report how long test organisms were acclimatized.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	The study says that "Responses are based on a sample size of 10" but it's unclear if that means 10 individuals or 10 brood cups (10 brood cups is recommended in the EPA effluent guidelines for C. dubia.) Elsewhere in the study it states "Newly prepared test solution and 24-h-old test solution composited from three replicates from each treatment level were analyzed." The methods say they follow the guidelines, but the description isn't explicit about how many animals were actually used.

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Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to *Ceriodaphnia dubia*. *Ecotoxicology and Environmental Safety* 39:136-146  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Only minor uncertainties about housing. "The standard, short-term, chronic toxicity test method developed for U.S. EPA's Whole Effluent Testing Program (U.S. EPA, 1994) was followed with modifications to minimize volatilization of test chemicals. Instead of 30-ml beakers, individual organisms were tested in 25-ml borosilicate glass vials filled to capacity and closed tightly using teflon PTFE-lined silicon septa held in place by polypropylene screw-on caps. These vials are sold by scientific suppliers as "EPA vials" for storage of water samples. Masten et al. (1994) found that static-renewal tests with these vials maintained concentrations of volatile chemicals more successfully than flowthrough test designs. Tests were conducted in artificial moderately hard water (U.S. EPA, 1994; Table 2). Light was provided by full spectrum fluorescent bulbs with a color rendering index >90 at an intensity of 20 mE/m <sup>2</sup> /S and a photoperiod of 16L: 8D. Daphnids were fed an algae and cereal leaf mix containing equal numbers of cells of <i>Scenedesmus capricornutum</i> and <i>Chlorella vulgaris</i> mixed with a rye grass infusion (ASTM, 1994). This mixture was added to diluted stock solutions to yield a final concentration of 3 x 10 <sup>5</sup> algal cells/ml and 0.03 mg/ml solids from cereal grass in each test vial. Component algae were cultured individually in modified Bold's basal medium (ASTM, 1994). Solutions were renewed daily. Dissolved oxygen was monitored on 24-h-old solutions and always remained above 7.0 ppm."
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	LC50 measured for acute toxicity over 48 hours
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences between study groups in environmental conditions or other factors that could influence the outcome assessment.

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Study Citation:	Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to <i>Ceriodaphnia dubia</i> . <i>Ecotoxicology and Environmental Safety</i> 39:136-146				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	707209				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	Table 4 indicates sample size for each test concentration, and shows if the sample size dropped to 9 rather than 10. All test conc for TCE had a sample size of 10.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	"Toxicity tests with individual chemicals were analyzed to determine criterion effect levels for mortality and reproduction following standard methods (U.S. EPA, 1994). Both 2- and 7-clay LC50s (concentrations lethal to 50% of the organisms) were calculated using binomial or trimmed Spearman-Kärber tests, as appropriate. After appropriate tests of normality, effects on reproduction (number of young per female through the end of the test) were analyzed with ANOVA, followed by one-sided Dunnett's comparison of means to the control to determine NOELs (no-observed effect levels) and LOELs (lowest-observed-effect levels). In addition, IC50s (concentrations inhibiting reproduction by 50 percent relative to mean control performance) were calculated from smoothed data using bootstrap analysis (Norberg-King, 1993)."
	Metric 22: Reporting of Data	High	× 2	2	Results were reported for each exposure group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes for TCE.
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. *Ecotoxicology and Environmental Safety* 39:136-146  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE identified by name
Metric 2:	Test Substance Source	High	× 1	1	Test compounds were obtained from Aldrich Chemical (Milwaukee, Wisconsin). All were widely employed industrial chemicals and have a mode-of-action classified as nonpolar narcosis (Schultz et al., 1990; Van Leeuwen et al., 1992; Russom et al., 1997).
Metric 3:	Test Substance Purity	Medium	× 1	2	Test substance purity is reported as 99.5 percent as labeled but not independently verified.
Domain 2: Test Design					
Metric 4:	Negative Controls	Low	× 2	6	Authors reported using negative controls but did not report details of the negative control group.
Metric 5:	Negative Control Response	High	× 1	1	Negative control response was reported in Table 4. No mortality was observed, and mean young for repro was reported.
Metric 6:	Randomized Allocation	Low	× 1	3	Not randomly allocated
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The test was conducted in a closed container and filled to capacity to limit volatilization. "25-ml borosilicate glass vials filled to capacity and closed tightly using teflon PTFE-lined silicon septa held in place by polypropylene screw-on caps. These vials are sold by scientific suppliers as "EPA vials" for storage of water samples. Masten et al. (1994) found that static-renewal tests with these vials maintained concentrations of volatile chemicals more successfully than flow through test designs."
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Only minor uncertainties about exposure administration.

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Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. Ecotoxicology and Environmental Safety 39:136-146  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	"In tests with individual components, toxicant concentrations were measured at the beginning and end of the experiment. Newly prepared test solution and 24-h-old test solution composited from three replicates from each treatment level were analyzed. [ . . . ] Chronic toxicological results are based on the geometric "mean of the four measured concentrations for each treatment level measured during the duration of the experiment."
	Metric 10: Exposure Duration and Frequency	High	× 1	1	7 days recommended for EPA effluent guidelines for C. dubia. <a href="https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf">https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf</a>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	EPA effluent guidelines recommend 5 test concentrations and 5 were used in this study. <a href="https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf">https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf</a>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Ceriodaphnia dubia is suitable and recommended in EPA effluent test guidelines. <a href="https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf">https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf</a>
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	The study not report how long test organisms were acclimatized.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	The study says that "Responses are based on a sample size of 10" but it's unclear if that means 10 individuals or 10 brood cups (10 brood cups is recommended in the EPA effluent guidelines for C. dubia.) Elsewhere in the study it states "Newly prepared test solution and 24-h-old test solution composited from three replicates from each treatment level were analyzed." The methods say they follow the guidelines, but the description isn't explicit about how many animals were actually used.

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Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to *Ceriodaphnia dubia*. *Ecotoxicology and Environmental Safety* 39:136-146  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 707209

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Only minor uncertainties about housing. "The standard, short-term, chronic toxicity test method developed for U.S. EPA's Whole Effluent Testing Program (U.S. EPA, 1994) was followed with modifications to minimize volatilization of test chemicals. Instead of 30-ml beakers, individual organisms were tested in 25-ml borosilicate glass vials filled to capacity and closed tightly using teflon PTFE-lined silicon septa held in place by polypropylene screw-on caps. These vials are sold by scientific suppliers as "EPA vials" for storage of water samples. Masten et al. (1994) found that static-renewal tests with these vials maintained concentrations of volatile chemicals more successfully than flowthrough test designs. Tests were conducted in artificial moderately hard water (U.S. EPA, 1994; Table 2). Light was provided by full spectrum fluorescent bulbs with a color rendering index >90 at an intensity of 20 mE/m <sup>2</sup> /S and a photoperiod of 16L: 8D. Daphnids were fed an algae and cereal leaf mix containing equal numbers of cells of <i>Scenedesmus capricornutum</i> and <i>Chlorella vulgaris</i> mixed with a rye grass infusion (ASTM, 1994). This mixture was added to diluted stock solutions to yield a final concentration of 3 x 10 <sup>5</sup> algal cells/ml and 0.03 mg/ml solids from cereal grass in each test vial. Component algae were cultured individually in modified Bold's basal medium (ASTM, 1994). Solutions were renewed daily. Dissolved oxygen was monitored on 24-h-old solutions and always remained above 7.0 ppm."
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	LC50, NOEC, LOEC, and IC50 (for repro) were measured after 7 days which is in accordance with EPA effluent guidelines for <i>C. dubia</i> . <a href="https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf">https://www.epa.gov/sites/production/files/2015-12/documents/method.1002.2002.pdf</a>
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
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Study Citation:	Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to <i>Ceriodaphnia dubia</i> . <i>Ecotoxicology and Environmental Safety</i> 39:136-146				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	707209				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences between study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	Table 4 indicates sample size for each test concentration, and shows if the sample size dropped to 9 rather than 10. All test conc for TCE had a sample size of 10.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	"Toxicity tests with individual chemicals were analyzed to determine criterion effect levels for mortality and reproduction following standard methods (U.S. EPA, 1994). Both 2- and 7-day LC50s (concentrations lethal to 50% of the organisms) were calculated using binomial or trimmed Spearman-Kärber tests, as appropriate. After appropriate tests of normality, effects on reproduction (number of young per female through the end of the test) were analyzed with ANOVA, followed by one-sided Dunnett's comparison of means to the control to determine NOELs (no-observed effect levels) and LOELs (lowest-observed-effect levels). In addition, IC50s (concentrations inhibiting reproduction by 50 percent relative to mean control performance) were calculated from smoothed data using bootstrap analysis (Norberg-King, 1993)."
	Metric 22: Reporting of Data	High	× 2	2	Results were reported for each exposure group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes for TCE.
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			
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Study Citation: Niederlehner, B., Cairns, J., Smith, E.. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to Ceriodaphnia dubia. Ecotoxicology and Environmental Safety 39:136-146  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 707209

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Labra, M.,Mattia, F.,Bernasconi, M.,Bertacchi, D.,Grassi, F.,Bruni, I.,Citterio, S.. 2010. The Combined Toxic and Genotoxic Effects of Chromium and Volatile Organic Contaminants to Pseudokirchneriella subcapitata. Water, Air, and Soil Pollution 213:57-70  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1059985

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name, trichloroethylene.
Metric 2:	Test Substance Source	High	× 1	1	Authors report that TCE is from Applichem, Germany.
Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	Low	× 2	6	There was a control, but authors reported only limited details.
Metric 5:	Negative Control Response	Low	× 1	3	The biological responses of the negative control groups were not reported.
Metric 6:	Randomized Allocation	Low	× 1	3	Authors did not comment on randomized allocation of test species.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	There was no mention of covering or sealing Erlenmeyer flasks, and it was not mentioned whether measurements were taken.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure administration seems consistent but not confirmed with measured concentrations. Only nominal concentrations used and there was no mention of flasks being covered.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	TCE is volatile and authors did not appear to measure the concentration in water.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	The study had a duration of 72 hours with a single exposure in Erlenmeyer flasks, which is in accordance with OECD 201 test guidelines.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Authors report 5 exposure groups, and at least 5 are suggested in OECD 201 test guidelines. Additionally, spacing of exposure groups were able to measure an effect.

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Study Citation:	Labra, M.,Mattia, F.,Bernasconi, M.,Bertacchi, D.,Grassi, F.,Bruni, I.,Citterio, S.. 2010. The Combined Toxic and Genotoxic Effects of Chromium and Volatile Organic Contaminants to Pseudokirchneriella subcapitata. Water, Air, and Soil Pollution 213:57-70				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	1059985				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Concentrations of TCE in this study were below TCE's solubility level.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Pseudokirchneriella subcapitata was used in this test, and is a recommended species in OECD 201 test guidelines.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Authors reported that the algae were acclimatized. " The P. subcapitata strain (SAG 61.81) used in this study was obtained from the Collection of Algal Cultures, G'ttingen, Germany (SAG). The algae were cultured in static and axenic condition in Bristol's medium solution (Nichols 1979). Algae were periodically transferred in a new medium (every 7 days) to obtain viable cell in log phase. These cultures were used to inoculate Erlenmeyer flasks containing 100 mL of solution to a final concentration of about 1"10 <sup>5</sup> cell mL"1."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	Algal cell concentrations were reported at about 1"10 <sup>5</sup> cell/mL which is within OECD 201 recommendations, and the test included two replicates.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Authors did not report all test conditions however controls seemed to do well, so only minor uncertainties remained.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	The authors described the outcome assessment methodology, reported results in the figures and the text, and the test was sensitive for the outcome of interest.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Study protocol was reported and outcomes were assessed consistently across study groups using the same protocol in all study groups.
Domain 6: Confounding / Variable Control					
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Study Citation:	Labra, M.,Mattia, F.,Bernasconi, M.,Bertacchi, D.,Grassi, F.,Bruni, I.,Citterio, S.. 2010. The Combined Toxic and Genotoxic Effects of Chromium and Volatile Organic Contaminants to Pseudokirchneriella subcapitata. Water, Air, and Soil Pollution 213:57-70				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	1059985				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among the study groups in environmental conditions of other factors that could influence the outcome of the assessment.
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among study groups that could influence the outcome of the assessment.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Authors reported acceptable statistical methods, and results of the analysis were reported for each study group. "Data were used to calculate mean values " standard deviations. Data were statistically analyzed by Statistica for Windows v. 6.0 (StatSoft Italia srl 2001) and StatGraphics programme for-Windows (version 5.0, Manugistic, MD, USA). The Duncan test for a multiple sample comparison was applied when normality and homogeneity of variance were satisfied."
	Metric 22: Reporting of Data	Medium	× 2	4	Some of the key data points were reported, but authors did not explicitly report a NOEC and a LOEC.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There were no unexplained outcomes reported.
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

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  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 1486051

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name (trichloroethylene).
Metric 2:	Test Substance Source	Low	× 1	3	The source of the chemicals were not identified.
Metric 3:	Test Substance Purity	Medium	× 1	2	Test purity was reported at at least 97 percent, but it is unclear exactly what purity TCE.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Study authors reported using a negative control but didn't say whether it was a water or solvent control.
Metric 5:	Negative Control Response	High	× 1	1	Negative control response was reported at always less than or equal to 10 percent.
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were assigned to study groups
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Closed system was used for TCE and maintenance of consistent exposure concentration was considered prime importance. Air spaces in exposure chambers were eliminated to minimize volatilization loss of the toxicant from water, a serious uncontrolled variable in many previous toxicity studies with hydrocarbons in static systems. This limited available oxygen to that dissolved in the water at the start of each test, typically at a concentration of 8-9 mg/l.
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Briefly, saturated aqueous solutions of single compounds were prepared and diluted to provide at least 5 exposure concentrations plus a control for each toxicity test. Test chambers were filled and sealed in 33 ml glass vials with teflon-lined screw caps.
Metric 9:	Measurement of Test Substance Concentration	Medium	× 2	4	Only nominal conc were used but measures were taken to limit test chemical loss in the test chambers.

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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  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 1486051

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	High	× 1	1	Static exposure for 24 hour duration for Artemia. Handbook of Ecotoxicology ed Calow (1998) says an LC50 can be obtained using Artemia after a 24 hour exposure.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Five exposure conc plus controls but unsure of the spacing of exposure levels.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	LC50 was well below TCE's solubility.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Artemia used
	Metric 14: Acclimatization and Pretreatment Conditions	Low	× 1	3	Study did not report whether organisms were acclimatized.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Number of organisms was not reported.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Handbook of Ecotoxicology ed Calow recommends 25 degrees in darkness for test procedures for this species. Temperature in this study may be too low. Study reports, Artemia nauplii were hatched from cysts under controlled environmental conditions to provide a large number of test organisms of exactly the same age and physiological condition for each test (Wells et al., 1982). Bioassays were conducted in the dark at 20 °C for Artemia tests. Animals were not feed, nor were toxicant solutions aerated during the 48 h exposure period. Checks on animal condition were made at 24 h and 48 h. Each animal was classified as alive/swimming or dead. Death was defined by the lack of visible internal or external movements after gently rotating the test chamber under a Stercostar zoom microscope.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome of interest was reported (LC50s).
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete details of test protocol was reported.

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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				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	1486051				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
Metric 19:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Unlikely to have major impacts on results.
Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition for each study group was not reported, but unlikely to have major impacts on results.
Domain 7: Data Presentation and Analysis					
Metric 21:	Statistical Methods	Medium	× 1	2	Percent mortality at each concentration was corrected for control mortality (always < or = to 10 percent) (Abbott, 1925) and nominal medial lethal concentrations (LC50 values), slope factors, and 95 percent confidence limits were calculated by the graphical method of Litchfield and Wilcoxon (1948).
Metric 22:	Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group, but results were described in the test. Data were only reported for some outcomes.
Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes reported.
Overall Quality Determination <sup>‡</sup>		Medium		1.8	
Extracted		Yes			

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Trichloroethylene identified by name
	Metric 2: Test Substance Source	High	× 1	1	Source is Merck 11872 p.a., lot no 8594665
	Metric 3: Test Substance Purity	Low	× 1	3	Purity was not reported for TCE.
Domain 2: Test Design					
	Metric 4: Negative Controls	Low	× 2	6	A control group was not reported but the authors say they did the test in accordance with OECD 305 which calls for a control.
	Metric 5: Negative Control Response	Low	× 1	3	Response was not reported
	Metric 6: Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	Low	× 2	6	It was a semi-static closed system that did have some head space. Authors admitted that a flow through design would be preferable with minimized headspace for volatile chemicals. The authors say that TCE in particular had considerable conc fluctuations in the various aquaria, and the guideline could not be followed in a meaningful way.
	Metric 8: Consistency of Exposure Administration	Unacceptable	× 1	4	The authors say that TCE in particular had considerable conc fluctuations in the various aquaria, and the guideline could not be followed in a meaningful way.
	Metric 9: Measurement of Test Substance Concentration	Medium	× 2	4	TCE was analyzed by gas chromatography with EC detector.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	The exposure was for 2 weeks according to OECD guideline 305 B. there were 8 aquaria each with 25 L capacity arranged and filled with 20 L deionized water. Stock solutions of test substances were prepared in water or acetone. Test conditions were semi static.

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Only one exposure group was used. The authors say, "The number of 7 samples per aquarium of 7 fish each sample, as proposed in the Guideline, could not be complied with, since, the oxygen content in the basins dropped exceedingly over 48 hours. The number of fish in the aquaria. was reduced to 28 or: 32 fish and, in general, a parallel experiment has been carried out. In the case of 28 fish on 7 consecutive days, in the case. of 32 fish on 8 consecutive days, 4 fish were sampled. In accordance with the Guideline, the work was carried out with two or three test concentrations, which differed by the factor of 10, whereby, as far as possible, the higher concentration was 1/50 below the EC-50 determined in this Institute."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism	Metric 13: Test Organism Characteristics	High	× 2	2	Zebra fish ( <i>Branchydanio rerio</i> ) were used which are a recommended species according to EPA test guidelines 850.1075.
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	Fish were acclimatized but uncertainties remain. Authors report, "Prior to test begin, the test fish were acclimatized in an 80 L aquarium. Unlike the test aquaria, this was continuously aerated. The water was reconditioned like in the test aquaria. The fish were fed several times daily with Tetra Min, using an automatic feeding device."

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	The study says, "The number of 7 samples per aquarium of 7 fish each sample, as proposed in the Guideline, could not be complied with, since, the oxygen content in the basins dropped exceedingly over 48 hours. The number of fish in the aquaria. was reduced to 28 or: 32 fish and, in 9eneral1, a parallel experiment has been carried out. In the case of 28 fish on 7 consecutive days, in the case. of 32 fish on 8 consecutive days, 4 fish were sampled. In accordance with the Guideline, the work was carried out with two or three test concentrations, which differed by the factor of 10, whereby, as far as possible, the higher concentration was 1/50 below the EC50 determined in this Institute."
	Metric 16: Adequacy of Test Conditions	High	× 1	1	After the fish were introduced the aquaria were closed with glass plates equipped with two no 29 standard ground apertures through which ground-glass thermometer and threaded pipe with HWS closure were introduced, the cable leading through the latter to an aquarium heater. the temperature in the aquaria were 23-25 degrees. After 48 hours samples were again taken the ph and the oxygen contents measured and then the fish were fed. Bout 2 hours after the feeding the fish were transferred into newly prepared aquaria, thus one cycle was complete.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	High	× 2	2	Average conc in water, average conc in fish, BCFs and duration were reported for each test conc. no information was provided for a control.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were addressed.
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Trichloroethylene identified by name
	Metric 2: Test Substance Source	High	× 1	1	Source is Merck 11872 p.a., lot no 8594665
	Metric 3: Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design					
	Metric 4: Negative Controls	Medium	× 2	4	Some information was given about a negative control, but minor uncertainties remain.
	Metric 5: Negative Control Response	Medium	× 1	2	A biological response in the control was alluded to when authors referred to the fact that growth for algae in this test was very slow after 3 days, so test results cannot be used.
	Metric 6: Randomized Allocation	Low	× 1	3	It was not reported whether a random allocation was used.
Domain 3: Exposure Characterization					

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 7: Experimental System/Test Media Preparation	Unacceptable	× 2	8	Growth of algae was very slow, so test criteria could not be satisfied, and tests were discontinued. "Since, in the case of low growth inhibition (approx. 1 - 15 percent), the growth curves for each concentration indicate very flat course, the EC10 values for all the chemicals tested could not be very accurately determine. Also the EC10 and EC50 values relating to the volatile substances, e.q. 1,1-dichloroethylene, trichloroethylene and benzene are uncertain. Since a large part of these chemicals volatilize, the EC10 and EC50 values could be lower than the values determined according to the guideline in Erlenmeyer flasks fitted with Kapsenberg caps. Therefore we have also carried out experiments in enclosed system on the basis of work published by Galassi and Vighi (7). Nevertheless, our experiments in 300 ml Erlenmeyer flasks fitted with ground-glass stopper indicated that the growth of algae was very slow and after 3 days stagnation set in. Since the test criteria could not be satisfied, these experiments were then discontinued."
	Metric 8: Consistency of Exposure Administration	High	× 1	1	
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	It is not clear whether measurements were taken but authors mention that volatile chemicals were at lower conc at the end of the test, alluding that they
	Metric 10: Exposure Duration and Frequency	High	× 1	1	the exposure was for 96 hours and in a static system, which is in accordance with EPA guidelines
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	This was not reported and results were not reliable according to study authors.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Fresh-water algae ( <i>Scenedesmus subspicatus</i> ) were used, which is in agreement with EPA test guideline 850.5400.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Pretreatment was not recorded.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	It is unclear how many organisms per exposure group.

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 16: Adequacy of Test Conditions	Unacceptable	× 1	4	Authors reported very slow growth in the algae, leading to authors discontinuing the test. "Since, in the case of low growth inhibition (approx. 1 - 15 percent), the growth curves for each concentration indicate very flat course, the EC10 values for all the chemicals tested could not be very accurately determine. Also the EC10 and EC50 values relating to the volatile substances, e.q. 1,1-dichloroethylene, trichloroethylene and benzene are uncertain. Since a large part of these chemicals volatilize, the EC10 and EC50 values could be lower than the values determined according to the guideline in Erlenmeyer flasks fitted with Kapsenberg caps. Therefore we have also carried out experiments in enclosed system on the basis of work published by Galassi and Vighi (7). Nevertheless, our experiments in 300 ml Erlenmeyer flasks fitted with ground-glass stopper indicated that the growth of algae was very slow and after 3 days stagnation set in. Since the test criteria could not be satisfied, these experiments were then discontinued."
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	

Domain 6: Confounding / Variable Control

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	Unacceptable	× 2	8	It seems that non-treatment-related factors may have influenced the results of the test. "Since, in the case of low growth inhibition (approx. 1 - 15 percent), the growth curves for each concentration indicate very flat course, the EC10 values for all the chemicals tested could not be very accurately determine. Also the EC10 and EC50 values relating to the volatile substances, e.q. 1,1-dichloroethylene, trichloroethylene and benzene are uncertain. Since a large part of these chemicals volatilize, the EC10 and EC50 values could be lower than the values determined according to the guideline in Erlenmeyer flasks fitted with Kapsenberg caps. Therefore we have also carried out experiments in enclosed system on the basis of work published by Galassi and Vighi (7). Nevertheless, our experiments in 300 ml Erlenmeyer flasks fitted with ground-glass stopper indicated that the growth of algae was very slow and after 3 days stagnation set in. Since the test criteria could not be satisfied, these experiments were then discontinued."
	Metric 20: Outcomes Unrelated to Exposure	Unacceptable	× 1	4	"Since, in the case of low growth inhibition (approx. 1 - 15 percent), the growth curves for each concentration indicate very flat course, the EC10 values for all the chemicals tested could not be very accurately determine. Also the EC10 and EC50 values relating to the volatile substances, e.q. 1,1-dichloroethylene, trichloroethylene and benzene are uncertain. Since a large part of these chemicals volatilize, the EC10 and EC50 values could be lower than the values determined according to the guideline in Erlenmeyer flasks fitted with Kapsenberg caps. Therefore we have also carried out experiments in enclosed system on the basis of work published by Galassi and Vighi (7). Nevertheless, our experiments in 300 ml Erlenmeyer flasks fitted with ground-glass stopper indicated that the growth of algae was very slow and after 3 days stagnation set in. Since the test criteria could not be satisfied, these experiments were then discontinued."

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Study Citation: Umweltbundesamt. 1987. ASSESSMENTS OF THE FEASIBILITY AND EVIDENCE OF TEST METHODS OF LEVELS I AND II OF THE CHEMICALS ACT ON THIOUREA.  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 1745587

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	Medium	× 2	4	Only EC10 and EC50 were reported
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were addressed
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			

\*\* Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, four of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Bacsi, I., Toeroek, T., B-Beres, V., Toeroek, P., Tothmeresz, B., Nagy, A. S., Vasas, G.. 2013. Laboratory and microcosm experiments testing the toxicity of chlorinated hydrocarbons on a cyanobacterium strain (*Synechococcus* PCC 6301) and on natural phytoplankton assemblages. *Hydrobiologia* 710:189-203

Data Type: Acute (0-96 hour); Aquatic; Plants

Hero ID: 2127844

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE was identified by name and described. Authors reported, "trichloroethylene (TCE)" one of the most widely produced chlorinated hydrocarbons"
Metric 2:	Test Substance Source	Low	× 1	3	not reported
Metric 3:	Test Substance Purity	Low	× 1	3	not reported
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	controls were used but details about what exactly controls included were not given. Authors reported, "The growth of the control cultures (without addition of chlorinated hydrocarbons) and treated cultures was monitored by measuring chlorophyll-a content and by counting cell numbers."
Metric 5:	Negative Control Response	Low	× 1	3	control response was given but only until 25 hours.
Metric 6:	Randomized Allocation	Low	× 1	3	not reported
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	the laboratory system was open and measurements were not taken, and flasks were open and aerated which can lead to rapid volatilization of TCE, however this was by design in order to better compare results in the lab to a microcosm experiment also performed.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	details not given about exposure administration for each exposure level.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	measured concentrations were not taken and cannot be expected to be close to nominal concentration due to the volatility of the chemical. However, this experiment measured effects in just the few hours after exposure.

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Study Citation: Bacsi, I.,Toeroek, T.,B-Beres, V.,Toeroek, P.,Tothmeresz, B.,Nagy, A. S.,Vasas, G.. 2013. Laboratory and microcosm experiments testing the toxicity of chlorinated hydrocarbons on a cyanobacterium strain (*Synechococcus* PCC 6301) and on natural phytoplankton assemblages. *Hydrobiologia* 710:189-203  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2127844

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	exposure happened once and was measured in the few hours after exposure. This is not in accordance with any guidelines, but was designed to mimic conditions that were carried out in microcosm experiment for comparison purposes.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	it appears only one exposure group was used to mimic the conditions in the microcosm
	Metric 12: Testing at or Below Solubility Limit	Low	× 1	3	unsure what the actual exposure concentration was from the author's reporting.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	cyanobacterium <i>Synechococcus elongatus</i> (PCC 6301). not a recommended test species in OECD 201 but in the same genus as a recommended test species for cyanobacteria
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	not reported
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	the initial cell density is outside the range for this genus in OECD201 ( <i>synechococcus leopoliensis</i> recommended cell density is 5x10 <sup>4</sup> -10 <sup>5</sup> ). This experiment starts at about 100x10 <sup>6</sup> . Each study was done in triplicate which is recommended.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	limited reporting of housing conditions
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Low	× 2	6	Outcome assessment methodology is described for changes in growth and enzyme activity. Growth measures are appropriate but some uncertainties remain for how enzyme activity was measured (with incomplete methodology described). Uncertainties also exist for when measures were taken. Measurements were taken for growth every second hour, and for enzyme activity at hour 0, 4, 8, 12, 24.
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	details regarding execution of study protocol across study groups was not reported.

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Study Citation: Bacsi, I.,Toeroek, T.,B-Beres, V.,Toeroek, P.,Tothmeresz, B.,Nagy, A. S.,Vasas, G.. 2013. Laboratory and microcosm experiments testing the toxicity of chlorinated hydrocarbons on a cyanobacterium strain (*Synechococcus* PCC 6301) and on natural phytoplankton assemblages. *Hydrobiologia* 710:189-203  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2127844

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	× 2	4	study did not provide enough information about env conditions across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	authors did not report data on health outcomes un-related to exposure
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Significance was determined trough ANOVA and Tukey test. All experiments were done in triplicate.
	Metric 22: Reporting of Data	Low	× 2	6	data was reported in figures, but not very well in text and the exact concentrations at which algae was exposed is not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were explained <i>e.g.</i> , "In laboratory cultures, chlorophyll content of the cells significantly changed only at the end of the experiments (Fig. 1c). Thus, the changes in the concentration of chlorophyll-a were because of the decrease in cell number. It is not clear if the toxicity of tetrachloro derivatives is higher. The membrane damaging effects of these lipophilic compounds, <i>i.e.</i> , because of their metabolism and oxidative stress can cause reduced chlorophyll-a levels. Such chlorophyll loss (decomposition of antenna pigments) was shown under oxidative stress in the green alga, <i>Chlorella vulgaris</i> (Qian et al., 2009)."
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			
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Study Citation: Bacsi, I.,Toeroek, T.,B-Beres, V.,Toeroek, P.,Tothmeresz, B.,Nagy, A. S.,Vasas, G.. 2013. Laboratory and microcosm experiments testing the toxicity of chlorinated hydrocarbons on a cyanobacterium strain (Synechococcus PCC 6301) and on natural phytoplankton assemblages. Hydrobiologia 710:189-203  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2127844

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, two of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Dobaradaran, S.,Mahvi, A. H.,Nabizadeh, R.,Ramavandi, B.,Nazmara, S.,Zarei, S.. 2012. BIOASSAY COMPARISON OF TRICHLOROETHYLENE (TCE) TOXICITY ON DAPHNIA MAGNA (D. MAGNA) BEFORE AND AFTER ULTRASOUND AND PHOTOLYSIS PROCESSES. Fresenius Environmental Bulletin 21:1533-1538

Data Type: Acute (0-96 hour); Aquatic; Invertebrates

Hero ID: 2127941

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE is identified by name and uses described
Metric 2:	Test Substance Source	High	× 1	1	Test source reported as Merck company, Germany.
Metric 3:	Test Substance Purity	High	× 1	1	The solutions were prepared by diluting the pure TCE (>99 percent), which was produced by Merck company, Germany.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	For bioassay experiments, 10 infants (age <24 h) were exposed to a volume of 100 ml in a 250-ml glass beaker (8 glass beakers for 8 different concentrations, and 1 beaker was considered as control in which TCE was not added).
Metric 5:	Negative Control Response	Low	× 1	3	Not reported
Metric 6:	Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	There was no mention that containers were covered, and no measurements were reported that would confirm exposure concentrations.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details of how exposures were administered were not reported, and it is important to know given TCE is volatile
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether measurements were taken
Metric 10:	Exposure Duration and Frequency	Low	× 1	3	There is no mention of renewing the TCE treated beakers with test chemical. Especially with volatile chemicals it would be better to have static renewal or flow through systems.

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Study Citation:	Dobaradaran, S.,Mahvi, A. H.,Nabizadeh, R.,Ramavandi, B.,Nazmara, S.,Zarei, S.. 2012. BIOASSAY COMPARISON OF TRICHLOROETHYLENE (TCE) TOXICITY ON DAPHNIA MAGNA (D. MAGNA) BEFORE AND AFTER ULTRASOUND AND PHOTOLYSIS PROCESSES. Fresenius Environmental Bulletin 21:1533-1538				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	2127941				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 8 different exposure levels, and OECD guideline 202 recommends at least 5. Authors report, "The initial concentration of TCE was 100 mg L <sup>-1</sup> , experimental concentrations tested were 100, 75, 50, 40, 30, 20, 10 and 5 percent of effluents from processes diluted with dilution water (ultrapure water, resistivity 18.2 M <sup>Ω</sup> cm)."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Highest test conc was 100 mg/L far below the solubility of TCE
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Daphnia magna used in this test
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Authors report, "Firstly, D. magna was caught from its living site, then cultured lonely, and infants of this primary D. magna were used for culture in large amounts. Dilution water was made up of tap water, which was dechlorinated by sodium thiosulfate, and also passed through a column of activated carbon. The quality parameters of dilution water are shown in Table 2. D. magna was maintained in a 10-L glass vessel containing medium for culture in a 12/12-h light-dark cycle, with temperature-control at 21 <sup>°</sup> C. Sheep manure was used as culture medium, and D. magna was fed with yeast every two days at a concentration of 100 mg L <sup>-1</sup> ."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	10 animals were placed in each beaker and the experiment was done in triplicate.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	10 L glass housing was used, with ph, hardness and temperature within the recommended ranges
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Medium	× 2	4	Methodology for counting lethality is good, however the recommended outcome to measure is immobilization which was not measured. Lethality was sensitive enough to see an effect for this experiment.

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Study Citation:	Dobaradaran, S.,Mahvi, A. H.,Nabizadeh, R.,Ramavandi, B.,Nazmara, S.,Zarei, S.. 2012. BIOASSAY COMPARISON OF TRICHLOROETHYLENE (TCE) TOXICITY ON DAPHNIA MAGNA (D. MAGNA) BEFORE AND AFTER ULTRASOUND AND PHOTOLYSIS PROCESSES. Fresenius Environmental Bulletin 21:1533-1538				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	2127941				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Consistent execution of the study.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Probit analysis was used to determine an LC50
	Metric 22: Reporting of Data	Low	× 2	6	Reporting of effects at each exposure level was great, but the actual exposure conc was reported in percentv/v, and measurements were not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Lukavsky, J.,Furnadzhieva, S.,Dittrt, F.. 2011. Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria. Bulletin of Environmental Contamination and Toxicology 86:226-231  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2128165

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical is identified by name and its uses are described.
Metric 2:	Test Substance Source	Low	× 1	3	The source was not reported.
Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Authors report using a control with methanol. Authors report, "Nutrient solution "Z", after Zehnder in Staub (1961), was spiked with TCE: 100 mg was dissolved in 2 mL of methanol (Lachema, CZ), and then added to 100 mL of sterile Z medium. Controls received an equal volume of methanol. This solution was diluted to the desired concentrations by an identical nutrient solution, but without either TCE or methanol."
Metric 5:	Negative Control Response	High	× 1	1	Negative control response was reported in figures and in the text.
Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation was not reported.
Domain 3: Exposure Characterization					
<b>Continued on next page . . .</b>					

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Study Citation: Lukavsky, J.,Furnadzhieva, S.,Dittrt, F.. 2011. Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria. Bulletin of Environmental Contamination and Toxicology 86:226-231  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2128165

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 7: Experimental System/Test Media Preparation	High	× 2	2	Authors took the volatility of TCE into consideration. Authors reported, "Assay in glass enclosures were performed according to ISO/DIS 14442 (1998) the glass enclosures (volume of 1.5 mL), were sealed with silicone stoppers coated with Teflon on the inner side (screw top vials, Supelco, USA). An identical nutrient solution was used, as described above, but also spiked with 3 g/L KHCO <sub>3</sub> (source of inorganic carbon). The inoculum of 0.04"0.06 g/L of dry weight was higher because of a shorter exposition time. The enclosures were exposed horizontally under the same light intensity and temperature as the plates and shaken 10 x/day. Every day, 0.2 mL was measured into 6 replicate wells in an immunological FB plate, and the OD 750 nm was measured identically as in the previous protocol. O <sub>2</sub> concentrations were measured by a polarographic Clark-type oxygen sensor, and pH by a miniaturized combined electrode, both joined to a MEM 102 multimeter (Chemoprojekt Satalice, CZ), the measurements were performed with no replicates."
	Metric 8: Consistency of Exposure Administration	Medium	× 1	2	Some species were tested in plates and some were tested in glass enclosures.
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	Measurements of TCE were not taken, however, authors reported measuring other conditions including "Every day, 0.2 mL was measured into 6 replicatewells in an immunological FB plate, and the OD 750 nm was measured identically as in the previous protocol. O <sub>2</sub> concentrations were measured by a polarographic Clarktype oxygen sensor, and pH by a miniaturized combinedelectrode, both joined to a MEM 102 multimeter (Chemoprojekt Satalice, CZ), the measurements were performed with no replicates."
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	The duration of the experiment were acceptable for algae, however authors did not indicate whether the experiment was static or static renewal.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 5 exposure groups which is OECD 201 recommended, and the concentrations were sensitive enough to derive an EC <sub>50</sub> .

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Study Citation: Lukavsky, J.,Furnadzhieva, S.,Dittrt, F.. 2011. Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria. Bulletin of Environmental Contamination and Toxicology 86:226-231  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2128165

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Conc were far below TCE's solubility level.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	At least one of the cyanobacteria species used are a recommended species in OECD201, <i>Desmodesmus subspicatus</i> . Other species used include <i>Raphidocelis subcapitata</i> and <i>Chlorella kessleri</i> .
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Acclimatization conditions were not reported.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	6 replicates were reported but no initial cell density was reported.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Temperature reported in study (30 degrees C) is higher than recommended temperature in OECD201 (21-24 +-2 degrees C). No temperature was given for the glass enclosures. It appears some of the health outcomes (related to growth) could have been due to housing conditions. However, part of the experiment was to determine whether the housing was adequate or not and they used two different housing conditions to compare.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Authors measured growth using dry weight. Authors indicate "OD in plates were evaluated under an iEMS reader (Labscale, SF) at wavelength 750 nm, and the optical density was converted to dry weight after individual conversion curves for each species. EC50 (the concentration of a tested substance at which the cell density, biomass, O2 production or pH increment is 50 percent of that of the untreated algae) was determined graphically according to standard ISO (2004) omitting."
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	There are minor uncertainties about consistency between outcome assessment between the dish and glass enclosure studies. Consider this minor because part of the purpose of the experiment was to determine which housing was more conducive to the experiment.

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Study Citation:	Lukavsky, J.,Furnadzhieva, S.,Dittrt, F.. 2011. Toxicity of Trichloroethylene (TCE) on Some Algae and Cyanobacteria. Bulletin of Environmental Contamination and Toxicology 86:226-231				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	2128165				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 6: Confounding / Variable Control					
Metric 19:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Only minor uncertainties. There was no mention of temperature in the glass enclosures. Both test setups were enclosed and sealed.
Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	It seemed some of the species were affected by the housing conditions.
Domain 7: Data Presentation and Analysis					
Metric 21:	Statistical Methods	High	× 1	1	Authors reported, "EC50 (the concentration of a tested substance at which the cell density, biomass, O2 production or pH increment is 50 percent of that of the untreated algae) was determined graphically according to standard ISO (2004) omitting the stimulation peaks. In addition, growth curves plotted as time" dry weight or OD 750, can indicate EC50 as concentration when cell number is constant, and stabilized. Six replicates were measured, for OD evaluation, error bars are plotted as " sx = H (nRx2 - (Rx)2/n2)."
Metric 22:	Reporting of Data	Medium	× 2	4	Some results were reported in detail for each exposure group. However, not all results were reported in this way
Metric 23:	Explanation of Unexpected Outcomes	Medium	× 1	2	Some of the unexpected outcomes were explained.
Overall Quality Determination <sup>‡</sup>		Medium		1.9	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Smith, A. D., Bharath, A., Mallard, C., Orr, D., Smith, K., Sutton, J. A., Vukmanich, J., McCarty, L. S., Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (*Jordanella floridae*). Archives of Environmental Contamination and Toxicology 20:94-102

Data Type: Acute (0-96 hour); Aquatic; Fish

Hero ID: 2298399

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name.
Metric 2:	Test Substance Source	Medium	× 1	2	The source of TCE was not reported, but gas chromatography was used to verify identity of chemical. "The determination of the test compounds in water samples was accomplished by solvent extraction followed by gas chromatography analysis."
Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Both water and acetone controls were used in acute static and flow-through tests
Metric 5:	Negative Control Response	Low	× 1	3	Control response was not reported
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report the method for how organisms were allocated to study groups, or their deficiencies regarding allocation method.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Static system with minimal headspace for volatile substance was used.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure concentrations were not reported in the static test
Metric 9:	Measurement of Test Substance Concentration	Medium	× 2	4	Static acute test didn't test concentrations and only relied on nominal concentrations.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	Static test was renewed every 24 hours over the course of 96 hours.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Concentrations were prepared in a logarithmic series but the method used to determine an appropriate range was not mentioned.
Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	

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Study Citation: Smith, A. D.,Bharath, A.,Mallard, C.,Orr, D.,Smith, K.,Sutton, J. A.,Vukmanich, J.,McCarty, L. S.,Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102

Data Type: Acute (0-96 hour); Aquatic; Fish

Hero ID: 2298399

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 4: Test Organism					
Metric 13:	Test Organism Characteristics	Medium	× 2	4	Juvenile flagfish (2-4 months) were used, and were laboratory raised. Not an OECD or EPA recommended species. Also had minor uncertainties about where the fish were obtained.
Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Minor uncertainties about details provided. Authors report, "Laboratory-reared juvenile (2-4 month) flagfish were used. Fish were raised in the diluent water and fed freshly-hatched and adult brine shrimp. Fish were not fed during the tests."
Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	Only 5 juvenile flagfish were used per aquarium when OECD recommends at least 7.
Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
Metric 17:	Outcome Assessment Methodology	High	× 2	2	
Metric 18:	Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	
Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	No adverse outcomes were reported for TCE, and control response was not reported.
Domain 7: Data Presentation and Analysis					
Metric 21:	Statistical Methods	High	× 1	1	
Metric 22:	Reporting of Data	Low	× 2	6	The data for the static test were not presented in full, and no information was reported for controls.
Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	There were no unexpected outcomes for TCE in this paper.

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Study Citation: Smith, A. D.,Bharath, A.,Mallard, C.,Orr, D.,Smith, K.,Sutton, J. A.,Vukmanich, J.,McCarty, L. S.,Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 2298399

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Smith, A. D., Bharath, A., Mallard, C., Orr, D., Smith, K., Sutton, J. A., Vukmanich, J., McCarty, L. S., Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (*Jordanella floridae*). Archives of Environmental Contamination and Toxicology 20:94-102

Data Type: Chronic (>21 days); Aquatic; Fish

Hero ID: 2298399

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name.
Metric 2:	Test Substance Source	Medium	× 1	2	The source of TCE was not reported, but gas chromatography was used to verify identity of chemical. "The determination of the test compounds in water samples was accomplished by solvent extraction followed by gas chromatography analysis."
Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Both water and acetone controls were used in chronic test
Metric 5:	Negative Control Response	High	× 1	1	Negative control response was reported for chronic test.
Metric 6:	Randomized Allocation	Medium	× 1	2	Researchers reported allocating fish randomly to the exposure apparatus. Did not specifically say if they were randomly allocated to control, but it is assumed, so only minor uncertainty.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The flow-through diluter was the same unit used in the acute
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure concentrations were reported for the chronic test. Five duplicate, logarithmically distributed concentrations of the test solutions, were used in 30-L aquaria.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Water samples were analyzed 5 days per week throughout the 28-day exposure period; sample collection was from the mid-point of the tanks. Determination of the test compounds in water samples was accomplished by solvent extraction followed by gas chromatography (GC) analysis.

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Study Citation:	Smith, A. D., Bharath, A., Mallard, C., Orr, D., Smith, K., Sutton, J. A., Vukmanich, J., McCarty, L. S., Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102				
Data Type:	Chronic (>21 days); Aquatic; Fish				
Hero ID:	2298399				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	High	× 1	1	Flow-through exposure with fresh solutions added at a rate of 6 L/hr.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	"Concentrations were prepared in a logarithmic series and the 96-hrLC50's calculated from the acute flagfish data were used to establish the exposure gradients employed in these chronic tests."
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Embryo/larval flagfish were used, and were laboratory raised. Not an OECD or EPA recommended species. Also had minor uncertainties about where the fish were obtained.
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	Minor uncertainties about details provided. Authors report, "Laboratory-reared juvenile (2-4 month) flagfish were used. Fish were raised in the diluent water and fed freshly-hatched and adult brine shrimp. Fish were not fed during the tests."
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	50 fry (one week old) per test level and the controls. Duplicate exposures were used, but OECD recommends 4 or 5.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	A 28-day outcome was measured which is what is recommended by OECD, but a 10-day outcome was also reported.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	
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Study Citation:	Smith, A. D.,Bharath, A.,Mallard, C.,Orr, D.,Smith, K.,Sutton, J. A.,Vukmanich, J.,McCarty, L. S.,Ozburn, G. W.. 1991. The acute and chronic toxicity of 10 chlorinated organic-compounds to the american flagfish (jordanella-floridae). Archives of Environmental Contamination and Toxicology 20:94-102				
Data Type:	Chronic (>21 days); Aquatic; Fish				
Hero ID:	2298399				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	No adverse outcomes were reported for TCE, and controls had 87 percent survival for a 28-day survival test.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	High	× 2	2	Control information was reported for outcomes.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There were no unexpected outcomes for TCE in this paper.
Overall Quality Determination <sup>‡</sup>		High		1.3	
Extracted		Yes			

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Rayburn, D. J. Deyoung, J. A. Bantle, D. J. Fort, R. Mcnew. 1991. Altered developmental toxicity caused by three carrier solvents. Journal of Applied Toxicology 11:253-260  
 Data Type: Other; Aquatic; other  
 Hero ID: 2307041

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	N/A		N/A	chemical identified by name, trichloroethylene, and by cas 79-01-6
	Metric 2: Test Substance Source	Medium	× 1	2	Source was reported, Aldrich chemical co, but batch/lot number was not reported and it was not reported whether the manufacturer verified the identity of the chemical or whether authors verified the identity through analytical methods.
	Metric 3: Test Substance Purity	Low	× 1	3	purity and or grade were not reported.
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	"Four groups of 25 embryos were exposed to FETAX solution and used as controls for each test."
	Metric 5: Negative Control Response	Medium	× 1	2	negative control response is reported in the text and in several tables. In at least one of the experiments the percent mortality was just over 10 percent.
	Metric 6: Randomized Allocation	Low	× 1	3	It was not reported whether animals were allocated randomly
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	Unacceptable	× 2	8	The study does not say whether petri dishes were covered or sealed in some way, and there were no concentrations measured to verify what the animals were exposed to.
	Metric 8: Consistency of Exposure Administration	Medium	× 1	2	
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	exposure concentrations were not reported.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	duration was 96 hour and every 24 hours solutions were renewed, which is the recommended duration and frequency from the ASTM Guidance.

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Study Citation: Rayburn, D. J. Deyoung, J. A. Bantle, D. J. Fort, R. Mcnew. 1991. Altered developmental toxicity caused by three carrier solvents. Journal of Applied Toxicology 11:253-260  
 Data Type: Other; Aquatic; other  
 Hero ID: 2307041

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	There were 6 exposure groups for TCE, but it is unclear how the spacing of exposure levels was determined, although it does appear as though they did a range finding test as they state, "One range and three definitive experiments were performed to determine the 96-h LC50, 96-h EC50 (malformation), the Teratogenic Index (TI) (96-h LCsc/96-h EC50) and the Minimum Concentration to Inhibit Growth (MCIG) for the three solvents and two teratogens. The 96-h LC25 and LCsci, and 96-h EC25 and EC50 (malformation) were determined using Litchfield-Wilcoxon probit analysis."
	Metric 12: Testing at or Below Solubility Limit	Medium	× 1	2	authors do not mention having trouble testing TCE at the levels they did, but with the way they reported the exposure levels percent(v/v) it's hard to tell whether the concs were below the solubility of TCE.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Xenopus embryos were used in these experiments which are the suggested species in ASTM guidance.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	study did not report whether the organisms were acclimatized. it only states, " Animal care and breeding were performed according toBantle et al. (1989)."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	two replicates per group with 20-25 embrayos used, which is according to the ASTM guidance.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Some information was given about housing, but not many details. Authors report adequate volume of solution in the Petri dishes which are plastic. Authors also say that each experiment folloed standard methods of test operation and embryo evaluation from two papers including Bantle et al (1989)and Dawson and Bangle (1987). However no specifics were given about temperaure, ph etc.
Domain 5: Outcome Assessment					

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Study Citation:	Rayburn, D. J. Deyoung, J. A. Bantle, D. J. Fort, R. Mcnew. 1991. Altered developmental toxicity caused by three carrier solvents. Journal of Applied Toxicology 11:253-260				
Data Type:	Other; Aquatic; other				
Hero ID:	2307041				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 17: Outcome Assessment Methodology	High	× 2	2	LC50 and EC50s were measured which were the suggested outcomes measured in the ASTM guidance, and the authors did measure NOELs and LOELs at the for mortality and malformations at the exposure levels tested.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	incomplete reporting of minor details
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	study did not report enough details to allow a comparison of environmental conditions across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	authors reported that at least one of the controls had a greater than 10 percent mortality and malformation rate, but it was just over 10 percent.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	"The 96-h LC25 and LCsci, and 96-h EC25 and EC50 (malformation) were determined using Litchfield-Wilcoxon probit analysis.20 Dunnett's test was used to determine the No-Observable-Effect Concentrations (NOEC) for malformation and mortality. The MCIG was determined by comparing head-tail lengths between control and experimental groups using the Hest for grouped observations."
	Metric 22: Reporting of Data	Unacceptable	× 2	8	results were reported in percent v/v which is not a concentration.
	Metric 23: Explanation of Unexpected Outcomes	Medium	× 1	2	explanations were given for unexpected outcomes.
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			
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Study Citation: Rayburn, D. J. Deyoung, J. A. Bantle, D. J. Fort, R. Mcnew. 1991. Altered developmental toxicity caused by three carrier solvents. Journal of Applied Toxicology 11:253-260  
 Data Type: Other; Aquatic; other  
 Hero ID: 2307041

Domain	Metric	Rating <sup>†</sup>	MWF* Score	Comments <sup>††</sup>
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\*\* Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, two of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Saotome, K., Tanaka, N., Takai, A., Sasaki, Y. F., Asano, N., Sofuni, T., Ojima, Y., 1998. Development of genotoxicity assay systems that use aquatic organisms. Mutation Research 399:125-133

Data Type: Other; Aquatic; Fish

Hero ID: 2800252

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name, trichloroethylene
Metric 2:	Test Substance Source	High	× 1	1	Source was identified by Wako Pure Chemicals, Osaka, Japan.
Metric 3:	Test Substance Purity	Low	× 1	3	Not reported
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Authors report a concentration of 0 for TCE in their tables of results. No control is mentioned in the text otherwise. Unsure if the control is just water or contains a solvent.
Metric 5:	Negative Control Response	High	× 1	1	Control response is noted in the tables
Metric 6:	Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Very little information is given about the actual exposure of the cells to TCE in this paper. It is unclear whether the volatile nature of TCE was taken into consideration during in the test setup to reduce volatilization. It also doesn't say anything about whether the source of the fish (collected periodically at Mochimune Harbor, Shizuoka prefecture, Japan) was contaminated or now.
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Very little information was given about how cells were exposed to TCE
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	The test substance was not measured and the test chemical is very volatile.
Metric 10:	Exposure Duration and Frequency	Low	× 1	3	Duration was 48 hours for this study. OECD guidance on genotoxicity assays say that 24 hours should be enough for assays without metabolic activation. It appears that there was only a one time exposure but it's unclear and no measurements were reported to determine the actual conc cells were exposed to.

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Study Citation:	Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Saotome, K., Tanaka, N., Takai, A., Sasaki, Y. F., Asano, N., Sofuni, T., Ojima, Y., 1998. Development of genotoxicity assay systems that use aquatic organisms. <i>Mutation Research</i> 399:125-133				
Data Type:	Other; Aquatic; Fish				
Hero ID:	2800252				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	6 exposure groups were used for this experiment
	Metric 12: Testing at or Below Solubility Limit	Medium	× 1	2	A subset of the exposure conc exceeded the water solubility limit but a sufficient range of exposure conc was tested to characterize a conc-response relationship. Just one conc (3000mg/L) was above the solubility level and there were 5 other conc levels below the solubility level.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	<i>R. ocellatus ocellatus</i> embryos were used for the chromosomal aberration. There are no guidelines for this type of study for fish. This species is suitable for metaphase analysis.
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	<i>Odontobutis obscura obscura</i> were collected from the Tomio River, Nara prefecture, Japan and then cultured in R. laboratory aquariums. Aquarium conditions were not reported.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Guidelines say that there should be enough cells and replicates at each exposure group to have statistical power. There were about 30 cells in each exposure level. However some conc levels had no replicates, some had duplicates and some had triplicates.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Housing was not described.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was partially reported by the authors but minor uncertainties remain.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	It appears that the outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among the study groups in environmental conditions that could influence the outcomes assessment.
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Study Citation:	Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinai, N., Saotome, K., Tanaka, N., Takai, A., Sasaki, Y. F., Asano, N., Sofuni, T., Ojima, Y.. 1998. Development of genotoxicity assay systems that use aquatic organisms. Mutation Research 399:125-133				
Data Type:	Other; Aquatic; Fish				
Hero ID:	2800252				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	Cell attrition reported for each study group and are acceptable.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	Fisher exact tests was used. The OECD genetic toxicology test guidelines state "the result is outside the distribution of the historical negative control data (e.g., Poisson-based 95 percent control limits)."
	Metric 22: Reporting of Data	Medium	× 2	4	Most data was presented in tables for each exposure level. Only minor uncertainties remain about how many replicates there were at higher exposure levels.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination <sup>‡</sup>		Medium		2.0	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2801609

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE is identified by name.
Metric 2:	Test Substance Source	Medium	× 1	2	Source was not reported but actual TCE test concentrations were analytically determined at the initiation and termination of the test or when 100 percent mortality occurred in a treatment.
Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Included a negative control group using just seawater.
Metric 5:	Negative Control Response	Low	× 1	3	Negative control response not reported.
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	The alga, <i>S. costatum</i> , was tested in 125-mL flasks containing 50 mL of test solution or control water. Each flask was inoculated with approximately 2.0 × 10 <sup>4</sup> cells/mL. The cultures were incubated at 20±1°C under 4,300 lux illumination. Test concentrations and controls were triplicated. Measurements of in vivo chlorophyll a were made using a Turner Model III fluorometer after 24, 48, 72, and 96 h of exposure. Cell counts were made after 96 h of exposure using a hemacytometer and Zeiss Standard 14-compound microscope. There was no mention of containers being covered, but the study did report test concentrations measured at the beginning and end of the test.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.

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Study Citation: Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 2801609

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	Test concentrations were measured at the beginning and end of the test.
	Metric 10: Exposure Duration and Frequency	High	× 1	1	The duration of exposure and frequency was reported as ok for algae. Could be 72 or 96 hours. In this case it was 96 hours.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	There were 5 exposure groups with a duplicate at each exposure level. no range finding test was conducted, and instead concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Skeletonema costatum is one of the recommended species for saltwater from EPA guidelines.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	"The alga tested was the chain-forming diatom; Skeletonemacostatum. The culture was obtained from the EPA Environmental Research Laboratory, Gulf Breeze, Fla., and maintained in stock culture at Bionomics Marine Research Laboratory (BMRL), Pensacola, Fla, according to procedures in U.S. EPA (1978)."
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Each flask was inoculated with approximately 2.0 x 10 <sup>4</sup> cells/mL (1.5 x 10 <sup>6</sup> is recommended by EPA guidelines for Skeletonema), and tests were done in triplicate which is recommended by EPA guidelines.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	The alga, S. costatum, was tested in 125-mL flasks containing 50 mL of test solution or control water. The cultures were incubated at 20±1°C under 4,300 lux illumination. This is recommended by EPA guidelines. Only minor uncertainties remain.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcomes of interest were measured appropriately.

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Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	2801609				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	The study did not provide enough information to allow a comparison of conditions or other non-treatment related factors across study groups.
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Based on the results of the tests, 24-, 48-, 72-, and 96-h LC50s or EC50s and 95 percent confidence limits were calculated, where possible. The computer program generated the LC- or EC50 values using the following statistical methods: moving average angle, probit, and binomial probability (Stephan 1977).
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related finding were not shown for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes reported.
Overall Quality Determination <sup>‡</sup>		Medium		1.8	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 2801609

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE is identified by name.
Metric 2:	Test Substance Source	Medium	× 1	2	Source was not reported but actual TCE test concentrations were analytically determined at the initiation and termination of the test or when 100 percent mortality occurred in a treatment.
Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Included a negative control group using just seawater.
Metric 5:	Negative Control Response	Low	× 1	3	Negative control response not reported.
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were allocated to study groups
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	The study used covered glass containers but volatilization still occurred in the static system. The study did report test concentrations measured at the beginning and end of the test.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Test concentrations were measured at the beginning and end of the test.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	The duration of exposure and frequency was reported as is appropriate for fish. 96 hour static test.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	There were 5 exposure groups with a duplicate at each exposure level. No range finding test was conducted, and instead concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.

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Study Citation:	Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	2801609				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Sheepshead minnow is one of the recommended species for saltwater from EPA guidelines (EPA TG 850.1085).
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	Some uncertainty around how acclimatization occurred. Study reports, "Sheepshead minnows, <i>Cyprinodon variegatus</i> , were hatched and reared for 4 to 6 days at BMRL. Sheepshead minnow eggs were spawned naturally in the laboratory in natural sea water basically following procedures in U.S. EPA (1978)."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	At least 7 fish per exposure group is recommended by OECD, and this test had 10.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Mysids and fish were tested in 1.6-L covered-glass dishes containing 1.0 L of test solution or control seawater. With 10 fish per container, and OECD recommended 1 liter per g of fish, and the average mass of fish were 1.4 mg we weight. Only small uncertainties about housing remain.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcomes of interest were measured appropriately
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	The study did not provide enough information to allow a comparison of conditions or other non-treatment related factors across study groups
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
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Study Citation:	Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	2801609				

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Based on the results of the tests, 24-, 48-, 72-, and 96-h LC50s or EC50s and 95 percent confidence limits were calculated, where possible. The computer program generated the LC- or EC50 values using the following statistical methods: moving average angle, probit, and binomial probability (Stephan 1977).
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related finding were not shown for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcome was explained, "Differences between TCE test concentrations at which mild intoxication symptoms were observed in test organisms in this study and by Borthwick (1977) may possibly be explained on the basis of carrier solvent synergism and/or temperature effects."
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 2801609

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	TCE is identified by name.
Metric 2:	Test Substance Source	Medium	× 1	2	Source was not reported but actual TCE test concentrations were analytically determined at the initiation and termination of the test or when 100 percent mortality occurred in a treatment.
Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Reported using a negative control group with just seawater.
Metric 5:	Negative Control Response	Low	× 1	3	Negative control response was not reported.
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	The study used covered glass containers but volatilization still occurred in the static system. The study did report test concentrations measured at the beginning and end of the test.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Test concentrations were measured at the beginning and end of the test.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	The duration of exposure and frequency was reported as is appropriate for mysids. 96 hour static test
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	There were 5 exposure groups with a duplicate at each exposure level. no range finding test was conducted, and instead concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.

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Study Citation:	Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	2801609				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were spaced by 6.25, 12.5, 25, 50 and 100 percent of the water soluble fraction.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Mysid shrimp is one of the recommended species for saltwater from EPA guidelines.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Study reports, "Mysid shrimp, <i>Mysidopsis bahia</i> , were born in culture at BMRL and maintained for 3 days before testing. Mysids were reared in natural sea water generally following procedures in U.S. EPA (1978). During holding, temperature was maintained at 22±1°C and salinity at 19 ppt."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	Ten mysids for each container and each concentration had a duplicate. EPA recommends 20 animals at each conc level (EPA TG 850.1035).
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Mysids and fish were tested in 1.6-L covered-glass dishes containing 1.0 L of test solution or control seawater. Shrimp were fed live (48-h old) brine shrimp nauplii on Days 0 and 2 during the test. Only small uncertainties about housing remain.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcomes of interest were measured appropriately.
	Metric 18: Consistency of Outcome Assessment	Medium	× 1	2	Incomplete reporting of minor details of outcome assessment protocol execution.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	The study did not provide enough information to allow a comparison of conditions or other non-treatment related factors across study groups
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis					
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Study Citation:	Ward, G. S., Tolmsoff, A. J., Petrocelli, S. R.. 1986. ACUTE TOXICITY OF TRICHLOROETHYLENE TO SALTWATER ORGANISMS. Bulletin of Environmental Contamination and Toxicology 37:830-836				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	2801609				

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 21: Statistical Methods	High	× 1	1	Based on the results of the tests, 24-, 48-, 72-, and 96-h LC50s or EC50s and 95 percent confidence limits were calculated, where possible. The computer program generated the LC- or EC50 values using the following statistical methods: moving average angle, probit, and binomial probability (Stephan 1977).
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related finding were not shown for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes were reported.
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Dierickx, P. J.. 1993. Comparison between fish lethality data and the in vitro cytotoxicity of lipophilic solvents to cultured fish cells in a two-compartment model. <i>Chemosphere</i> 27:1511-1518					
Data Type:	Acute (0-96 hour); Aquatic; Fish					
Hero ID:	2803221					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
Domain 1: Test Substance						
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name, trichloroethylene	
Metric 2:	Test Substance Source	Low	× 1	3	Not reported	
Metric 3:	Test Substance Purity	Low	× 1	3	Purity, grade not reported	
Domain 2: Test Design						
Metric 4:	Negative Controls	Medium	× 2	4	A control was used, but not much detail is given. Authors report, "During this period the cells, remaining in culture medium, are in contact with the test chemical, solubilized in paraffin, through the microporous membrane. Pure paraffin was used as the control."	
Metric 5:	Negative Control Response	Low	× 1	3	Not reported	
Metric 6:	Randomized Allocation	Low	× 1	3	Allocation method not reported	
Domain 3: Exposure Characterization						
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	TCE is very volatile, and there was no mention of covering the wells or preventing loss of test substance. However the test was only 24 hours long so some of the TCE may have been in the wells by the end of the test.	
Metric 8:	Consistency of Exposure Administration	High	× 1	1		
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Exposure conc was not measured or not reported	
Metric 10:	Exposure Duration and Frequency	Medium	× 1	2	The exposure duration was 24 hours, which was enough time to record an effect of total protein inhibition, so it appears to be a sufficient duration. Only minor uncertainties remain. It was a static exposure in wells.	
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Number of exposure groups and spacing were not reported; however the authors were able to determine an EC50 for total protein expression.	
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Study Citation: Dierickx, P. J.. 1993. Comparison between fish lethality data and the in vitro cytotoxicity of lipophilic solvents to cultured fish cells in a two-compartment model. Chemosphere 27:1511-1518  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 2803221

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 12: Testing at or Below Solubility Limit	Medium	× 1	2	Because exposure levels were not reported, it is unclear if they were all below the solubility limit, however the EC50 was far below the solubility for TCE.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Cultured fathead minnow fish cells were used for this study. While this is a well studied species, minor uncertainties remain about whether this cell line (FHM cells American Type Culture Collection N <sup>o</sup> CCI42 established fishcell line, derived from tissue posterior to the anus from fathead minnow) are appropriate for this type of in vitro test.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Cells were cultured in the lab and pretreated before the test.
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Authors report, "6.105 cells in 0.2 mL culture medium were seeded in each of the 12 transwells." Minor uncertainties about what cell density is appropriate for fish cells.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Control response was not reported for TCE so it's difficult to tell whether the housing conditions were adequate for this cell line's normal functioning
Domain 5: Outcome Assessment					
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Study Citation: Dierickx, P. J.. 1993. Comparison between fish lethality data and the in vitro cytotoxicity of lipophilic solvents to cultured fish cells in a two-compartment model. Chemosphere 27:1511-1518  
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 Hero ID: 2803221

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 17: Outcome Assessment Methodology	High	× 2	2	EC50s (for total protein inhibition) were determined to measure cytotoxicity using the following methodology, "Each well was rinsed once with 0.5 mL Hank's balanced salt solution on the outer membranous side and twice on the inner side, and placed in an empty well of a new 24well tissue culture plate. The remaining cells were lysed with 0.2 mL 2 percent Na2CO3 in 0.1 N NaOH per transwell for 15 min at 34 °C. This solution was then replaced 2 times with the same solution, at room temperature, and the combined fractions were used for a protein determination according to Lowry et al. (1951) as previously described for Hep G2 cells (Dierickx, 1989). The results are expressed as percentages of control cultures, which typically contained 85-105 µg protein/transwell. The relative toxicity of the test chemicals is established by the determination of the EC50. This is the concentration of test chemical in the lower compartment required to induce a 50 percent inhibition of the total protein content in the upper compartment (transwell). In order to determine the reproducibility of EC50 determinations, the EC50 values of cyclopentanol, cyclohexanone and 2-chlorotoluene were measured in three independent assays. A standard deviation of less than 6 percent was found in these independent assays. Therefore, the EC50 values of the other compounds were determined in single experiments."
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	The study did not provide enough information to allow a comparison of the environmental conditions for each study group.
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Cell attrition was not reported for TCE for each study group.
Domain 7: Data Presentation and Analysis					
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Study Citation:	Dierickx, P. J.. 1993. Comparison between fish lethality data and the in vitro cytotoxicity of lipophilic solvents to cultured fish cells in a two-compartment model. Chemosphere 27:1511-1518				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	2803221				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 21: Statistical Methods	Low	× 1	3	The statistical method used to derive the EC50 was not reported in detail. Authors simply state, "Interpolation of these curves gives the EC50 values, by computing the concentration needed to reduce the total protein content by 50 percent."
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings were not reported for each study group.
	Metric 23: Explanation of Unexpected Outcomes	Low	× 1	3	Authors acknowledge the discrepancy between this study (which did not find a linear correlation between the published LC50 levels and the EC50 values they produced in this test) and previous studies which did, but the reasons are unclear.
Overall Quality Determination <sup>‡</sup>		Low		2.3	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Bacsi, I.,Gonda, S.,B-Beres, V.,Novak, Z.,Nagy, S. A.,Vasas, G.. 2015. Alterations of phytoplankton assemblages treated with chlorinated hydrocarbons: effects of dominant species sensitivity and initial diversity. <i>Ecotoxicology</i> 24:823-834					
Data Type:	Acute (0-96 hour); Aquatic; Plants					
Hero ID:	3298076					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
Domain 1: Test Substance						
Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, trichloroethylene, and use was described.	
Metric 2:	Test Substance Source	Low	× 1	3	Not reported	
Metric 3:	Test Substance Purity	Low	× 1	3	Not reported	
Domain 2: Test Design						
Metric 4:	Negative Controls	Medium	× 2	4	Controls in beakers and pond sample controls were used but details about what exactly controls included were not given. Authors reported, "12 l water sample from the pond was filled into 4 plastic (polimethylpenthene"PMP) beakers (3 l to each one)."	
Metric 5:	Negative Control Response	Medium	× 1	2	Control response was reported in figures, until 3 days. Some uncertainties remain about exact numbers for control response, but an approximation can be seen in the figures.	
Metric 6:	Randomized Allocation	N/A		N/A	Not applicable to allocate individual algae to study groups randomly.	
Domain 3: Exposure Characterization						
Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Beakers were used for the pond experiment and the authors allude the fact that the beakers are "enclosed", but it is unclear whether enough precautions are taken to avoid volatilization of the test chemicals and no measurements of test chemical were taken. No nominal concentrations were given either.	
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details not given about exposure administration for each exposure level.	
<b>Continued on next page ...</b>						

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Study Citation: Bacsı, I.,Gonda, S.,B-Beres, V.,Novak, Z.,Nagy, S. A.,Vasas, G.. 2015. Alterations of phytoplankton assemblages treated with chlorinated hydrocarbons: effects of dominant species sensitivity and initial diversity. *Ecotoxicology* 24:823-834  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 3298076

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	Measured concentrations were not taken and cannot be expected to be close to nominal concentration due to the volatility of the chemical. Additionally this experiment measured effects 24 hours, 48 hours and 96 hours after exposure, giving this substance plenty of time to volatilize.
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	96 hours is an acceptable amount of time to measure effects in algae however the exposure only occurred once at time 0, and this chemical volatilizes quickly, so multiple exposures are necessary to maintain test concentrations.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	N/A		N/A	It appears only one exposure group was used however, with a microcosm experiment this may be acceptable.
	Metric 12: Testing at or Below Solubility Limit	Low	× 1	3	Unsure what the actual exposure concentration was from the author's reporting. No measurements were taken to confirm, but authors report, "Treated assemblages were theoretically saturated solvents at the beginning of the experiments."
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Test organisms were a variety of algae species (59 taxa were reported in 2011 and 95 in 2012) found in the Garden Pond in the Botanical Garden of the University of Debrecen. Dominant species was <i>Trachelomonas volvocinopsis</i> .
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Not reported
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Authors report, "All experiments were done in triplicates," which is recommended. Abundance was reported at about $11 \times 10^6$ in the 2012 experiment, which is outside the range of densities given in OECD 201 recommendations (e.g., the high-est recommended cell density is for <i>synechococcus leopoliensis</i> at $5 \times 10^4$ - $10^5$ ).
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Beakers were plastic and it is unclear whether they are chemically inert. Measurements of pH, temperature, O <sub>2</sub> conc were taken.

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Study Citation: Bacsı, I.,Gonda, S.,B-Beres, V.,Novak, Z.,Nagy, S. A.,Vasas, G.. 2015. Alterations of phytoplankton assemblages treated with chlorinated hydrocarbons: effects of dominant species sensitivity and initial diversity. *Ecotoxicology* 24:823-834  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 3298076

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology is described for changes in growth, and species diversity. Growth measures are appropriate according OECD guidance 201. Diversity was measured in the following way, "The phytoplankton samples were processed according to the Utermo"hl method (1958) and European Standard EN 15204:2006. An Olympus CKX31 inverted microscope and 4009 magnification was used for counting and taxa identification."
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	Details regarding execution of study protocol across study groups was not reported.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	Authors measured ph, O2 conc, water temp, and contols and treatment groups were kept in similar conditions in the pond in the same basket.
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	A storm could have affected growth, diversity in this experiment.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	Significance was determined trough ANOVA and Tukey test. All experiments were done in triplicate.
	Metric 22: Reporting of Data	Low	× 2	6	Data was reported in figures, but not very well in text and the exact concentrations at which algae was exposed is not reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexpected outcomes were addressed, "The observed decrease of chlorophyll-a content and algal abundance in the first 24 h probably occurred due to a summer storm with stormy wind, rain and a temperature drop of 6 C in 2011. Meteorological events like heavy rainfall, hot weather periods and stormy hours can have a pronounced impact on hydraulics, water temperature and nutrient supply. Such short-term variability is thought to be important in the changes of algal community structure."

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Study Citation: Bacsı, I.,Gonda, S.,B-Beres, V.,Novak, Z.,Nagy, S. A.,Vasas, G.. 2015. Alterations of phytoplankton assemblages treated with chlorinated hydrocarbons: effects of dominant species sensitivity and initial diversity. *Ecotoxicology* 24:823-834  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 3298076

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Houde, M., Douville, M., Gagnon, P., Sproull, J., Cloutier, F.. 2015. Exposure of <i>Daphnia magna</i> to trichloroethylene (TCE) and vinyl chloride (VC): evaluation of gene transcription, cellular activity, and life-history parameters. <i>Ecotoxicology and Environmental Safety</i> 116				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	3502953				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
	Metric 1: Test Substance Identity	High	× 2	2	Chemical identified by name and cas
	Metric 2: Test Substance Source	Medium	× 1	2	Source of TCE was reported but not analytically verified. Authors report, "Stock standard solutions of TCE was obtained from Supelco (Bellefonte, PA, USA) and were diluted in methanol and water."
	Metric 3: Test Substance Purity	Low	× 1	3	Not reported
Domain 2: Test Design					
	Metric 4: Negative Controls	High	× 2	2	Methanol control group was included with 6 replicates. methanol is listed as a suitable control solvent for daphnia, and authors report that methanol concentration never exceeded 0.1 percent which is recommended in OECD 211 as well.
	Metric 5: Negative Control Response	Medium	× 1	2	Control response was reported for some but not all responses. <i>e.g.</i> , no control response was reported for gene expression.
	Metric 6: Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
	Metric 7: Experimental System/Test Media Preparation	High	× 2	2	Physical chemical properties were taken into consideration. Authors used standardized methods for volatile organic compounds (PNLET, 2011). Each beaker was covered with a watch glass and left at 20 degrees C.
	Metric 8: Consistency of Exposure Administration	High	× 1	1	A lot of detail was reported in the paper and supplementary information about exposure administration timing and actual concentrations. Exposure administration was described as
	Metric 9: Measurement of Test Substance Concentration	High	× 2	2	Authors measured conc of TCE extensively, reporting the measured conc at hour 0 1 2 3 4 5 6 8 24 30 48.
<b>Continued on next page . . .</b>					

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Study Citation:	Houde, M., Douville, M., Gagnon, P., Sproull, J., Cloutier, F.. 2015. Exposure of <i>Daphnia magna</i> to trichloroethylene (TCE) and vinyl chloride (VC): evaluation of gene transcription, cellular activity, and life-history parameters. <i>Ecotoxicology and Environmental Safety</i> 116				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	3502953				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	High	× 1	1	Considering the volatile properties of the studied compounds, renewal of the media was conducted every 24 h in order to ensure the continuous exposure of <i>D. magna</i> . New stock solutions were prepared at every media renewal and water temperature, conductivity, dissolved oxygen, pH, and hardness were monitored.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	For the chronic toxicity test, only 3 conc were tested, and OECD 211 recommends 5. Concentrations were determined by what levels found in the environment (in river water in Quebec Canada).
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Concentrations were far below TCE's solubility.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	<i>Daphnia magna</i> were used in this study
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Pretreatment conditions were reported and acceptable. Authors reported, "Genetically homogenous <i>D. magna</i> were cultured in growthchamber following Environment Canada's method (Environment Canada, 1990). Cultures were kept at 20/21 °C with a photoperiod of 16h light:8h dark. Organisms were fed green algae <i>Pseudokirchneriella subcapitata</i> (concentration: 3.85105 cells/mL) and YCT preparation (yeast "cerophyll" trout chow, concentration: 0.0125g/L) everyday. All experiments were performed under the same constant temperature and diurnal lighting conditions."
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	OECD recommends 10 animals at each test conc. In this study 10 animals were used per treatment.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Temperature, light/dark period were within OECD 211 recommended ranges. Food was also appropriate.
Domain 5: Outcome Assessment					

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Study Citation:	Houde, M., Douville, M., Gagnon, P., Sproull, J., Cloutier, F.. 2015. Exposure of <i>Daphnia magna</i> to trichloroethylene (TCE) and vinyl chloride (VC): evaluation of gene transcription, cellular activity, and life-history parameters. <i>Ecotoxicology and Environmental Safety</i> 116				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	3502953				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Outcome was measured by protein expression and genes being up or down regulated. No EC50 was established, and no effect on overall reproduction was found.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	Test setup and procedure seems sufficient
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	No unexpected health outcomes
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	Most of the statistical analysis mentioned were appropriate (recommended in OECD 211, with only some uncertainty around using the Kruskal-wallis test for brooding time. Authors report, "Daphnia brooding times were compared with Kruskal-Wallis tests. Numbers of neonates were modeled as over dispersed Poisson variates and animal sizes at the end of the 10 days exposure were compared with ANOVAs. Relative gene transcription between exposed and non-exposed organisms (methanol controls) were compared using Wilcoxon signed ranks tests (probabilities corrected to control the false discovery rate). Biomarker responses to contaminant concentrations were compared to controls with ANOVAs and Dunnett's procedure. Relationships between biomarkers and genes were assessed using Spearman's correlations. The significance threshold was established at 0.05. All statistical analyses were conducted using SAS9.4 (SAS Institute Inc.).
	Metric 22: Reporting of Data	Medium	× 2	4	Data was reported for each study group for some but not all end points.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Sufficient discussion of results

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Study Citation: Houde, M., Douville, M., Gagnon, P., Sproull, J., Cloutier, F.. 2015. Exposure of *Daphnia magna* to trichloroethylene (TCE) and vinyl chloride (VC): evaluation of gene transcription, cellular activity, and life-history parameters. *Ecotoxicology and Environmental Safety* 116

Data Type: Other; Aquatic; Invertebrates

Hero ID: 3502953

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Overall Quality Determination <sup>‡</sup>		High		1.3	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Vidal, M., Bassères, A., Narbonne, J.. 2001. Potential biomarkers of trichloroethylene and toluene exposure in *Corbicula fluminea*. *Environmental Toxicology and Pharmacology* 9:87-97  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 3546158

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Substance was identified by name
Metric 2:	Test Substance Source	Low	× 1	3	Not reported
Metric 3:	Test Substance Purity	Low	× 1	3	Not reported
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Controls consisted in two aquariums each containing 10 l of filtered lake water. Aquariums were covered to limit water and/or product evaporation.
Metric 5:	Negative Control Response	High	× 1	1	Response of the negative control was reported
Metric 6:	Randomized Allocation	Low	× 1	3	Not reported
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	The loss of TCE during the preparation of the contaminated water and during the exposure was very important in spite of glass covers on aquariums and moderate, intermittent aeration to try to reduce evaporation. Measurements were taken to verify the actual conc the clams were exposed to. Results of chemical analysis are presented in Table 1. As TCE and TOL are volatile products, an important loss was observed during the preparation of mixtures, due mainly to homogenisation (loss of 23 up to 44 percent for TCE and 6 up to 45 percent for TOL).
Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Some loss of test chemical during preparation of mixtures but in general the administration was consistent being a one-time static exposure.
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Measurements of test substances were taken; nominal conc were 1.56, 6.25, 25 and 100 mg/l for TCE and measured conc were 1.2, 3.6, 14, and 69.4.

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Study Citation:	Vidal, M., Bassères, A., Narbonne, J.. 2001. Potential biomarkers of trichloroethylene and toluene exposure in <i>Corbicula fluminea</i> . Environmental Toxicology and Pharmacology 9:87-97				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	3546158				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 10: Exposure Duration and Frequency	Medium	× 1	2	Exposure was a one-time static exposure with tox results assessed at the end of 5 days. The guidelines for acute tox for bivalves from EPA suggest a 48 hour flow through exposure, but authors in this paper were trying to mimic conditions that may occur in the field with a one time spill.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	5 tests concentrations are recommended in EPA test guidelines for bivalves (850.1055). this test only has 4 test concentration
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Test conc were far below TCE solubility level.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	<i>Corbicula fluminea</i> , a freshwater clam, is not a listed recommended test species for bivalves in the EPA 850.1055 guidelines but is present in freshwater environments in Asia, the US, and increasingly in Europe.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15: Number of Organisms and Replicates per Group	Medium	× 1	2	Replicates not reported; number of organisms was reported.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Temperature, ph, light and dark, lack of crowded conditions are consistent with what is recommended for the
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	There does not seem to be any differences among study groups in environmental conditions.
	Metric 20: Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis					
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Study Citation: Vidal, M., Bassères, A., Narbonne, J.. 2001. Potential biomarkers of trichloroethylene and toluene exposure in *Corbicula fluminea*. *Environmental Toxicology and Pharmacology* 9:87-97  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 3546158

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 21:	Statistical Methods	High	× 1	1	Authors report, "Statistica software (5.1 release, Statsoft) was used for all statistical evaluations. Data distributed normally were submitted to a Brown-Forsythe test for homogeneity of variances (across dose groups). Data fulfilling this requirement were then submitted to a univariate analysis of variance (ANOVA) with "dose" (of TCE or TOL) as the independent variable and biochemical parameters as dependent variables. Whenever a significant (P50.05) dose effect was established on a biochemical parameter response, a Tukey HSD multiple comparison test was used to check significant (P50.05) differences between doses. Correlations between the dose and biochemical parameter responses were determined with the Pearson correlation coefficient (significant for P50.05). As additional studies, discriminant analysis (DA), often referred to as canonical variate analysis, were performed to try to discriminate doses (of TCE or TOL) from each other and to determine biochemical parameters allowing the best discrimination between doses. Unlike univariate analysis, multivariate analysis such as DA takes into account the whole biochemical parameter responses and, thus, provides an integrated approach. DA procedures and its applications in environmental biomonitoring have been described by Adams et al. (1994)."
Metric 22:	Reporting of Data	Medium	× 2	4	Mechanistic data was reported for each exposure level, however behavioral patterns mentioned in the discussion were not reported and would be helpful in establishing an EC50.
Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			

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Study Citation: Vidal, M., Bassères, A., Narbonne, J.. 2001. Potential biomarkers of trichloroethylene and toluene exposure in *Corbicula fluminea*.  
 Environmental Toxicology and Pharmacology 9:87-97  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 3546158

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Ando, T.,Otsuka, S.,Nishiyama, M.,Senoo, K.,Watanabe, M. M.,Matsumoto, S.. 2003. Toxic Effects of Dichloromethane and Trichloroethylene on the Growth of Planktonic Green Algae, *Chlorella vulgaris* NIES227, *Selenastrum capricornutum* NIES35, and *Volvoxella steinii* NIES545. 18:43-46

Data Type: Other; Aquatic; Plants

Hero ID: 3617103

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name, trichloroethylene, and described as a typical organic solvent.
Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not specified.
Metric 3:	Test Substance Purity	Low	× 1	3	The purity not specified.
Domain 2: Test Design					
Metric 4:	Negative Controls	High	× 2	2	Controls were included for each species.
Metric 5:	Negative Control Response	High	× 1	1	The growth effects were reported for the controls in the figures.
Metric 6:	Randomized Allocation	Low	× 1	3	There was no mention of random allocation.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	According to the authors description, this testing procedure appeared to be static, but details about the test procedure were lacking. In particular, there was uncertainty about whether evaporation of the solvents occurred.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across study groups.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Test concentrations reported in terms of nominal concentrations.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	The test algae were cultured for 10 days and the absorption of light by chlorophyll a was measured every 2 days as a measure of algal growth.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Concentrations were reported as 0, 0.003, 0.03, 0.3 and 3 mg/L. This corresponded to 0, one-tenth, tenfold and 100 fold of the Japanese national effluent standards for TCE. However, there were only 4 test concentrations while OECD recommends at least 5.

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Study Citation:	Ando, T.,Otsuka, S.,Nishiyama, M.,Senoo, K.,Watanabe, M. M.,Matsumoto, S.. 2003. Toxic Effects of Dichloromethane and Trichloroethylene on the Growth of Planktonic Green Algae, <i>Chlorella vulgaris</i> NIES227, <i>Selenastrum capricornutum</i> NIES35, and <i>Volvulina steinii</i> NIES545. 18:43-46					
Data Type:	Other; Aquatic; Plants					
Hero ID:	3617103					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Test conc. were well below TCE's solubility level.	
Domain 4: Test Organism						
	Metric 13: Test Organism Characteristics	High	× 2	2	C. vulgaris, S. capricornutum, and V. Steinii were used for this study. Strains obtained from Japanese national collection. Although only one species is an OECD recommended test organisms, the other organisms were chosen to cover species that would be able to grow in a wide range of water qualities.	
	Metric 14: Acclimitization and Pretreatment Conditions	Medium	× 1	2	Algae were incubated prior to addition of test material, but details were not provided, so minor uncertainties remain.	
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	Authors indicated 5 replicates in this study, and OECD 201 recommends at least 3.	
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Test vessels were consistent with recommendations for algal growth potential (AGP) test.	
Domain 5: Outcome Assessment						
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Absorbance as a measure of growth/biomass was reported for all test concentrations, which can be used as a surrogate for biomass when measured by a spectrophotometer (as it was in the study) according to OECD TG 201.	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Outcomes we assessed consistently across study groups.	
Domain 6: Confounding / Variable Control						
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	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	× 1	2	Data on attrition or health outcomes unrelated to exposure were not reported for each study group but are not likely to have substantial impacts on results.	
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Study Citation:	Ando, T.,Otsuka, S.,Nishiyama, M.,Senoo, K.,Watanabe, M. M.,Matsumoto, S.. 2003. Toxic Effects of Dichloromethane and Trichloroethylene on the Growth of Planktonic Green Algae, <i>Chlorella vulgaris</i> NIES227, <i>Selenastrum capricornutum</i> NIES35, and <i>Volvulina steinii</i> NIES545. 18:43-46
Data Type:	Other; Aquatic; Plants
Hero ID:	3617103

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Low	× 1	3	Statistical analysis was performed to determine the significance of differences between control and test concentrations, but test methods were not presented.
	Metric 22: Reporting of Data	Low	× 2	6	Absorbance as a measure of growth/biomass was reported for all test concentrations, which can be used as a surrogate for biomass when measured by a spectrophotometer (as it was in the study) according to OECD TG 201. And relative absorbance was reported for each species and each study group in the figures. However, no EC50 was derived for the species that did see an effect on relative absorbance.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes reported.
Overall Quality Determination <sup>‡</sup>		Medium		1.7	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Yoshioka, Y.,Ose, Y.,Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 3617749

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name: Trichloroethylene.
Metric 2:	Test Substance Source	Low	× 1	3	Source of TCE was not reported, but it was noted that analytical grade TCE was used.
Metric 3:	Test Substance Purity	Medium	× 1	2	Purity not reported but it was noted that analytical grade TCE was used.
Domain 2: Test Design					
Metric 4:	Negative Controls	Low	× 2	6	The study refers to a blank but doesn't say what's in the blank. I assume this is the control for D. japonica (planarian).
Metric 5:	Negative Control Response	Low	× 1	3	The study reports that most of the planarian in the blank test regenerated heads normally, but a number isn't given and TCE isn't discussed specifically.
Metric 6:	Randomized Allocation	Low	× 1	3	It's not reported whether animals were randomly allocated.
Domain 3: Exposure Characterization					

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Study Citation: Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21  
 Data Type: Other; Aquatic; Invertebrates  
 Hero ID: 3617749

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	It's unclear whether the experiment was conducted in a closed or open system using static or flow through methods. The study reports, "The breeding liquid for <i>Dugesia japonica</i> was prepared by dissolving 3.74 g of NaCl, 0.49 g of KCl, and 8.55 g of CaCl <sub>2</sub> into distilled water to make 500 ml. This was diluted 100 times and neutralized by NaHCO <sub>3</sub> before use. <i>Dugesia japonica</i> were collected from a stream around which there was no source of pollution and left without food for over 7 days in the breeding liquid to excrete alimentary canal contents. Those of about .2 cm long were used. <i>Dugesia japonica</i> was cut into two parts (head and body part) at the nearest section to the eyes of the trisected part between pharynx and eyes. The body part was used for the head regeneration test. Ten body parts were put in 100 ml of a test solution, and this was left at 20 °C for 7 days. Observation for head regeneration was carried out with a stereomicroscope on Days 3, 4, 5, 6, and 7 after head cutting, and the test solution was replaced at every observation. The degree of regeneration was classified as normal, eye spot, tetratophthalmic, anophthalmic, aciphthalmic, and death. The total number of eye spot, tetratophthalmic, anophthalmic, aciphthalmic, and death was regarded as the abnormal regeneration number. The ratio of the number to 10 on Day 7 was defined as the abnormal regeneration rate. The concentration of the chemical, at which the abnormal regeneration rate reached 50 percent, was defined as EC50." LC50 of <i>D. japonica</i> was determined at the same time. LC50 and EC50 values of the test mentioned above were determined on semilogarithmic paper."
Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure methods were not reported for each study group
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether nominal or measured conc were used.
Metric 10:	Exposure Duration and Frequency	Medium	× 1	2	Exposure occurred over 7 days, and observation was carried out on days 3, 4, 5, 6, and 7 after head cutting, and the test solution was replaced at every observation.

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Study Citation: Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21					
Data Type: Other; Aquatic; Invertebrates					
Hero ID: 3617749					
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Not reported for TCE, but for other chemicals it looks like 4 exposure groups were used plus control.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Substance was tested well below solubility.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Low	× 2	6	Uncertainties about the quality of the test organisms given they were collected from the field and no acclimation is mentioned. Study reports, "Dugesia japonica were collected from a stream around which there was no source of pollution and left without food for over 7 days in the breeding liquid to excrete alimentary canal contents. Those of about 2 cm long were used."
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Did not report whether they were acclimatized and they were collected from the field.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	The number of animals in each solution was not clear, possibly ten? The study says "Ten body parts were put in 100 ml of a test solution, and this was left at 20 °C for 7 days." Is this 10 body parts from 10 different individuals? Number of replicates not reported.
	Metric 16: Adequacy of Test Conditions	Low	× 1	3	Housing not mentioned for planarian.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Determined an LC50
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	Details of outcome assessment were not reported.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Confounding variables are discussed for planarian. The study says that confounding may occur due to the cutting of the head (stress of cutting of the head).
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Data on health and attrition were not reported for each study group.
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Study Citation:	Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21				
Data Type:	Other; Aquatic; Invertebrates				
Hero ID:	3617749				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	Methods not described clearly
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings not reported for each study group
	Metric 23: Explanation of Unexpected Outcomes	Medium	× 1	2	Authors did report unexpected outcomes and explained some of them, including the planarian numbers being very different than the other two species.
Overall Quality Determination <sup>‡</sup>		Low		2.5	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21					
Data Type:	Acute (0-96 hour); Aquatic; Fish					
Hero ID:	3617749					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
Domain 1: Test Substance						
	Metric 1: Test Substance Identity	High	× 2	2	Chemical identified by name: Trichloroethylene.	
	Metric 2: Test Substance Source	Low	× 1	3	Source of TCE was not reported, but it was noted that analytical grade TCE was used.	
	Metric 3: Test Substance Purity	Low	× 1	3	Purity was not reported.	
Domain 2: Test Design						
	Metric 4: Negative Controls	Unacceptable	× 2	8	The study does not mention a control anywhere. The study refers to a blank for <i>Dugesia japonica</i> (planarian) but doesn't say what's in the blank, and doesn't mention a blank for <i>O. latipes</i> (red killifish).	
	Metric 5: Negative Control Response	N/A		N/A	No control reported	
	Metric 6: Randomized Allocation	Low	× 1	3	It's not reported whether animals were randomly allocated.	
Domain 3: Exposure Characterization						
	Metric 7: Experimental System/Test Media Preparation	Medium	× 2	4	Test was completed in a closed container (sealed with an electrode), but there were some uncertainties about how much air space there was in the flask.	
	Metric 8: Consistency of Exposure Administration	Low	× 1	3	Exposure methods were not reported for each study group.	
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether nominal or measured conc were used.	
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	Exposure occurred over 48 hours, and it sounds like a static test but it is not clear. OECD recommends 96 hours for fish acute tests.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	For TCE, it is unclear how many exposure groups were used for the LC50 determination. (For the oxygen uptake it looks like 5 exposure groups according to figure 2 but that was a different test. )	
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Substance was tested well below solubility.	
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Study Citation: Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 3617749

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	Medium	× 2	4	Minor uncertainties about the quality of the test organisms given they were collected from the market. Study reports, "Orizias latipes (ca. 3 cm, 0.3 g) was obtained from the market and acclimated for at least 1 week in dechlorinated water at 20°C (total hardness was about 80 mg/liter). LC50 was determined by exposing 10 O. latipes to 2 liters of a chemical solution at 20 °C for 48 hr with the cycle 8 hr dark and 16 hr light. The oxygen uptake rate was determined by putting 10 O. latipes in an Erlenmeyer flask (3-liter) filled with test solution which was saturated with air, and the flask was sealed with an electrode. Then it was left without aeration at 20°C for 4 hr. The concentration of dissolved oxygen (DO) was measured by a DO meter (Denkikagaku type 3) every 30 min. As the oxygen was not supplied by aeration during the test, the result was accepted only when DO concentration was over 3 mg/liter at the end of the test in order to avoid the influence of the lack of DO. If DO decreased to under 3 mg/liter, the test was carried out anew with 5 O. latipes. After the test, the wet weight of O. latipes was measured in order to calculate the oxygen uptake rate per wet weight."
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	× 1	2	Fish were acclimatized for 1 week and OECD recommends 12 days before they are used for testing.
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	10 organisms per exposure group. OECD recommends at least 7
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	10 fish in 2 liters of water which is a little more than what OECD would recommend. At 0.3 g each and 10 fish per container, it should be a 3 liter flask.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Derived an LC50
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	Details of outcome assessment were not reported.
Domain 6: Confounding / Variable Control					
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Study Citation:	Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21					
Data Type:	Acute (0-96 hour); Aquatic; Fish					
Hero ID:	3617749					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	Study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups, and the omitted information is likely to have a substantial impact on study results.	
	Metric 20: Outcomes Unrelated to Exposure	Low	× 1	3	Data on health and attrition were not reported for each study group.	
Domain 7: Data Presentation and Analysis						
	Metric 21: Statistical Methods	Medium	× 1	2	Methods not described clearly.	
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings not reported for each study group.	
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes for the killifish.	
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0		
Extracted		No				

\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation:	Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21					
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates					
Hero ID:	3617749					
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>	
Domain 1: Test Substance						
	Metric 1: Test Substance Identity	High	× 2	2	Chemical identified by name: Trichloroethylene.	
	Metric 2: Test Substance Source	Low	× 1	3	Source of TCE was not reported, but it was noted that analytical grade TCE was used.	
	Metric 3: Test Substance Purity	Low	× 1	3	Purity is not reported.	
Domain 2: Test Design						
	Metric 4: Negative Controls	Unacceptable	× 2	8	The study does not mention a control anywhere. The study refers to a blank for <i>Dugesia japonica</i> (planarian) but doesn't say what's in the blank, and doesn't mention a blank for <i>O. latipes</i> (red killifish).	
	Metric 5: Negative Control Response	N/A		N/A	No control reported	
	Metric 6: Randomized Allocation	Low	× 1	3	It's not reported whether animals were randomly allocated.	
Domain 3: Exposure Characterization						
	Metric 7: Experimental System/Test Media Preparation	Low	× 2	6	It is not reported whether the container was closed or open, and TCE is a volatile chemical.	
	Metric 8: Consistency of Exposure Administration	Low	× 1	3	Exposure methods were not reported for each study group.	
	Metric 9: Measurement of Test Substance Concentration	Low	× 2	6	It was not reported whether nominal or measured conc were used.	
	Metric 10: Exposure Duration and Frequency	Low	× 1	3	Exposure occurred over 4 hours, and OECD 202 recommends 48 hours for invertebrate acute tests.	
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	For TCE, it is unclear how many exposure groups were used for the LC50 determination.	
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	Substance was tested below solubility.	
Domain 4: Test Organism						
<b>Continued on next page . . .</b>						

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Study Citation:	Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21				
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates				
Hero ID:	3617749				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 13: Test Organism Characteristics	Low	× 2	6	Test species is a saltwater invertebrate, and were used at 5 days old, but the source of the species is not reported.
	Metric 14: Acclimitization and Pretreatment Conditions	Low	× 1	3	Study did not report acclimating water fleas.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	Ten organisms per exposure group. For freshwater invertebrates, OECD 202 recommends at least 20.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	"Ten <i>M. macrocopa</i> in 100 ml of test solution were put in a 250-ml vial vessel at 20 °C and the survivors were counted after 3 hr in order to determine LC50."
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Derived an LC50
	Metric 18: Consistency of Outcome Assessment	Low	× 1	3	Details of outcome assessment were not reported.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	Low	× 2	6	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	Low	× 1	3	Data on health and attrition were not reported for each study group.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	Medium	× 1	2	Methods not described clearly.
	Metric 22: Reporting of Data	Low	× 2	6	Data for exposure related findings not reported for each study group.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexplained outcomes for the water flea.
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			
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Study Citation: Yoshioka, Y., Ose, Y., Sato, T.. 1986. Correlation of the Five Test Methods to Assess Chemical Toxicity and Relation to Physical Properties. 12:15-21  
 Data Type: Acute (0-96 hour); Aquatic; Invertebrates  
 Hero ID: 3617749

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
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\*\* Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, two of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Tsai, K. P., Chen, C. Y.. 2007. An Algal Toxicity Database of Organic Toxicants Derived by a Closed-System Technique. Environmental Toxicology and Chemistry 26:1931-1939  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 3617867

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name. Physical chemical characteristics such as MW, LogP, HLC, and solubility were provided for the test material.
Metric 2:	Test Substance Source	Low	× 1	3	The source was not provided.
Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not provided. Authors described the chemical purity as "reagent grade."
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Authors referred to a control when discussing how they calculated their EC50 value, but additional details were not reported. The authors indicated that the details of the test setup can be found at the following source: Lin JH, Kao WC, Tsai KP, Chen CY. 2005. A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants. Water Res 39:1869-1877. This source indicates that inclusion of a negative control is a part of the testing procedure.
Metric 5:	Negative Control Response	Low	× 1	3	Negative Control response was not specifically reported in the study, but was incorporated into the calculation of the percent inhibition.
Metric 6:	Randomized Allocation	Low	× 1	3	Researchers did not report how organisms were allocated to study groups
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The goal of the study authors was specifically to determine the optimal testing conditions for algae.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	The goal of the study authors was specifically to determine the optimal testing conditions for algae by providing a more consistent exposure regime.

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Study Citation: Tsai, K. P., Chen, C. Y.. 2007. An Algal Toxicity Database of Organic Toxicants Derived by a Closed-System Technique. Environmental Toxicology and Chemistry 26:1931-1939  
 Data Type: Acute (0-96 hour); Aquatic; Plants  
 Hero ID: 3617867

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 9: Measurement of Test Substance Concentration	Medium	× 2	4	Test concentrations were reported in terms of nominal concentrations, but analytical confirmation of the test concentrations was performed at the beginning and end of the test by HPLC. This was intended to quantify any potential degradation.
	Metric 10: Exposure Duration and Frequency	Medium	× 1	2	Authors reported, "All tests were conducted in triplicate, with a test duration of 48 h. The population density of the algae was determined using an electronic particle counter" 48 hours is acceptable, but 72 hours is recommended in OECD 201.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	The study report indicated that both a range finding and definitive test were conducted but did not report the test concentrations.
	Metric 12: Testing at or Below Solubility Limit	Low	× 1	3	It is unclear what test conc were, but the solubility of TCE is very high (999-1472 mg/l), and the EC50 determined was relatively low in comparison 26.24 mg/l)
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Green algae ( <i>Pseudokirchneriella subcapitata</i> ) which is a recommended test species in OECD Guideline 201.
	Metric 14: Acclimitization and Pretreatment Conditions	High	× 1	1	Supplemental methodology indicates that algae are incubated until a steady-state is reached
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	3 Replications were used and cell density was and the initial inoculated cell density was 15,000 cells/ml. which is in the OECD 201 recommended range of 5*10 <sup>3</sup> -10 <sup>4</sup> for this species.
	Metric 16: Adequacy of Test Conditions	High	× 1	1	Temperature and light intensity were kept at 24 1C and 65 Em2s1 (10 percent), respectively. Algal growth medium [22] with no ethylenediaminetetraacetic acid content was used for toxicity testing. The initial pH for the growth medium was 7.5, and the initial inoculated cell density was 15,000 cells/ml.
Domain 5: Outcome Assessment					

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Study Citation:	Tsai, K. P., Chen, C. Y.. 2007. An Algal Toxicity Database of Organic Toxicants Derived by a Closed-System Technique. Environmental Toxicology and Chemistry 26:1931-1939				
Data Type:	Acute (0-96 hour); Aquatic; Plants				
Hero ID:	3617867				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Authors proved that closed-system algae testing is more conservative than batch testing. Were able to derive an EC50.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups using the same protocol.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	The purpose of the experiment was to determine whether algae are more sensitive to volatile chemicals when tested in closed-systems than in batch testing. They confirmed that closed system tests were more sensitive for algae relative to batch tests.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition was not reported for each study group, but is unlikely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	The EC50 was determined using probit analysis.
	Metric 22: Reporting of Data	Medium	× 2	4	Results did not include effects at each concentration level; however, this is unlikely to substantially influence outcomes.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There were no unexpected outcomes.
Overall Quality Determination <sup>‡</sup>		High		1.6	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Schell, J. D. J.. 1987. Interactions of Halogenated Hydrocarbon Mixtures in the Embryo of the Japanese Medaka (*Oryzias latipes*).  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 3625489

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical is identified by name
Metric 2:	Test Substance Source	High	× 1	1	Purchased from Fisher scientific
Metric 3:	Test Substance Purity	High	× 1	1	Study reports TCE is certified ACS which I believe means greater than 99.5 percent.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Clean rearing solution was used as a control, with only minor uncertainties about formulation.
Metric 5:	Negative Control Response	High	× 1	1	
Metric 6:	Randomized Allocation	Low	× 1	3	Did not report whether allocation to study groups was random.
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Volatility of chemical was taken into account in the study design. "Since the chemicals of interest are volatile, exchanging solutions on a daily basis insured a more consistent exposure level."
Metric 8:	Consistency of Exposure Administration	High	× 1	1	
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations were used and were not measured. TCE is volatile. Rate of loss was determined for carbon tet and chloroform, but not TCE.
Metric 10:	Exposure Duration and Frequency	High	× 1	1	10 day exposure duration (96 hour preferred for acute, and 28 day preferred for chronic, so this exposure period is sub-chronic) which is recommended for fish early life stage OECD. Static renewal method used. Fresh test solutions were exchanged in the vials each day for 10 consecutive days.
Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	After a range finding test, TCE concentrations were 54, 59, 65, 72, 79, and 87 mg/l (log-dose intervals). 6 concentrations used plus control; OECD recommends at least 5 for fish early life stage.
Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	

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Study Citation: Schell, J. D. J.. 1987. Interactions of Halogenated Hydrocarbon Mixtures in the Embryo of the Japanese Medaka (*Oryzias latipes*).  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 3625489

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 4: Test Organism					
Metric 13:	Test Organism Characteristics	High	× 2	2	rice fish (Japanese Medaka)
Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	"Adult male and female medaka used for breeding purposes were obtained from the Carolina Biological Supply Company (Burlington, N.C.). Upon arrival in the laboratory, fish were quarantined from existing laboratory stocks and treated for 24 h with a mixture of malachite green (0.05 mg/L) and formalin (20 mg/L) to protect against infection resulting from the stress associated with handling and transportation during shipment. Following treatment, the fish were partitioned into breeding stocks at a ratio of 3 males:2 females with an optimum number of 60 fish per JO gallon aquarium."
Metric 15:	Number of Organisms and Replicates per Group	Medium	× 1	2	10 embryos per dose group, which is acceptable, but no mention of how many replicates.
Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
Metric 17:	Outcome Assessment Methodology	High	× 2	2	
Metric 18:	Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among the study groups in environmental conditions or other factors that could be influencing the outcome assessment.
Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition was reported in each exposure group. Other health outcomes were not reported, but I consider these only minor uncertainties.
Domain 7: Data Presentation and Analysis					
Metric 21:	Statistical Methods	High	× 1	1	
Metric 22:	Reporting of Data	Medium	× 2	4	Most but not all outcomes were reported. only minor uncertainties.

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Study Citation: Schell, J. D. J.. 1987. Interactions of Halogenated Hydrocarbon Mixtures in the Embryo of the Japanese Medaka (*Oryzias latipes*).  
 Data Type: Other; Aquatic; Fish  
 Hero ID: 3625489

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Unexplained outcomes were reported and discussed for the other chemicals in this paper. No unexplained outcomes were noted for TCE.
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name, trichloroethylene, and by CAS no. 79-01-6.
Metric 2:	Test Substance Source	Medium	× 1	2	Unclear where TCE specifically was from. Study reports, "Almost all test chemicals were purchased from Aldrich Chemical (Milwaukee, WI, USA), with only a few supplied by other commercial companies. Regardless of source, all chemicals were of a high level of purity ( <i>i.e.</i> , 95 percent or greater) and were not repurified before testing."
Metric 3:	Test Substance Purity	Medium	× 1	2	At least 95 percent purity but unclear what the purity of TCE is specifically.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Controls were used, and assume it was just a lake water control given the study says "no solvent carriers were used and all toxicants were tested at concentrations below their reported solubility."
Metric 5:	Negative Control Response	Low	× 1	3	No response was reported for control
Metric 6:	Randomized Allocation	Medium	× 1	2	Concentrations were distributed randomly between the two diluters, and for the acute test it was not mentioned whether animals were distributed randomly amongst control groups, but study did report that procedures followed standard American Society for Testing and Materials experimental procedure and as described previously. For the chronic experiment, embryos were added five at a time into 56 different egg cups which then randomly were added to the treatment and control chambers so that each tank received one cup.
Domain 3: Exposure Characterization					

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Study Citation: Broderius, S. J., Kahl, M. D., Elonen, G. E., Hammermeister, D. E., Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	Flow through test was conducted, which is better for volatile chemicals like TCE. It was not specifically noted whether the containers were covered and how much headspace was allowed, but the flow through nature of the test should help with keeping TCE conc consistent. Also measured concentrations were taken.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Continuous flow through with a check for any spikes. See following description "To verify the accuracy of the method of analyses, known amounts of toxicant were added to control water so that toxicant concentrations could be corrected routinely for spiked water recoveries. At least one spike sample was prepared for each sample set. A mean percent recovery of 90 to 110 percent usually was observed. All statistical/model analyses were based on measured concentrations."
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	"Methods of chemical analysis included high-pressure liquid chromatography with ultraviolet or diode array/fluorescence detectors, and gasliquid chromatography with flame ionization or electron capture detectors. Samples of all test solutions were taken from the test chambers and toxicant mixing trays and analyzed according to a monitoring program that characterized the toxicant exposures. Duplicate measurements were made routinely with each analytical series to define the reproducibility (precision) of the measurements. Percentage duplicate agreements usually were greater than 90 percent. To verify the accuracy of the method of analyses, known amounts of toxicant were added to control water so that toxicant concentrations could be corrected routinely for spiked water recoveries. At least one spike sample was prepared for each sample set. A mean percent recovery of 90 to 110 percent usually was observed. All statistical/model analyses were based on measured concentrations."
Metric 10:	Exposure Duration and Frequency	High	× 1	1	Continuous exposure for 96 hours which is one recommended option for the fish acute guidelines from OECD

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Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Acute (0-96 hour); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Authors used 5 exposure groups, and at least 5 is recommended by OECD 203 test guidelines.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	TCE concentrations tested were below the solubility of TCE.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Fathead minnow are an acceptable test species according to OECD 203 test guidelines.
	Metric 14: Acclimatization and Pretreatment Conditions	High	× 1	1	Acclimatization took place, and fish were cultured in the lab with appropriate temp for fathead minnow, and ph of 7.8 was within the range of OECD 203 test guidelines. Water hardness was also in the range of OECD 203 test guidelines.
	Metric 15: Number of Organisms and Replicates per Group	Low	× 1	3	7 fish are recommended per test conc. It is unclear how many fish were used in the acute test from the description in this paper, "The acute toxicity tests to determine 96-h LC50 values were conducted according to a standard American Society for Testing and Materials experimental procedure [28] and as described previously [27]. By exposing groups of test organisms to a range of concentrations in continuous flow-through systems for separate, binary, or equitoxic multiple chemical mixtures, the percentage mortality in 96-h was determined for numerous tests. Toxicity tests were conducted initially with individual toxicants and expanded subsequently to numerous binary test solutions and multiple toxicant mixtures. All isoboles of acute joint toxic action were determined for the 96-h LC50 response level. A plot of 96-h LC50 values and 95 percent confidence limits was constructed as test data allowed for each binary mixture.

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow ( <i>Pimphales promelas</i> ). 24:3117-3127					
Data Type: Acute (0-96 hour); Aquatic; Fish					
Hero ID: 3665276					
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Housing for the most part are acceptable, but test water was obtained from lake superior and underwent sand filtration. There could be other contaminants in the water, however because controls were used, and chemicals measurement and analysis took place, only minor uncertainties regarding this detail. The appropriate temp for fathead minnow was used, and the ph of 7.8 within the range of OECD 203 test guidelines. Water hardness was also in the range of OECD 203 test guidelines.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	LD50 was measured for acute toxicity. EC50 and EC20 were measured and determined for growth for chronic toxicity.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent between study groups.
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences in study groups from environmental conditions of other factors that could influence the outcome of assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes were not reported for each study group
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	In the 96-h toxicity tests, mortalities were recorded daily, and an estimate of the LC50 and its 95 percent confidence limits was determined by the trimmed Spearman-Karber method [32]. The 96-h LC50s from the binary mixtures were used to construct isobole diagrams of joint toxic action for the 50 percent3120 Environ. Toxicol. Chem. 24, 2005 S.J. Broderius et al. response level. The data on combined effects of binary mixtures were analyzed by comparison of the observed results with that expected for the concentration- or response-addition models.
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Study Citation:	Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow ( <i>Pimphales promelas</i> ). 24:3117-3127				
Data Type:	Acute (0-96 hour); Aquatic; Fish				
Hero ID:	3665276				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 22: Reporting of Data	Medium	× 2	4	Data were not reported for outcomes with negative findings, but tis is unlikely to have substantial impact on results.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	There were no unexplained outcomes.
Overall Quality Determination <sup>‡</sup>		High		1.5	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rfloor_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.

Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Chronic (>21 days); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name, trichloroethylene, and by CAS no. 79-01-6.
Metric 2:	Test Substance Source	Medium	× 1	2	Unclear where TCE specifically was from. Study reports, "Almost all test chemicals were purchased from Aldrich Chemical (Milwaukee, WI, USA), with only a few supplied by other commercial companies. Regardless of source, all chemicals were of a high level of purity ( <i>i.e.</i> , 95 percent or greater) and were not repurified before testing."
Metric 3:	Test Substance Purity	Medium	× 1	2	At least 95 percent purity but unclear what the purity of TCE is specifically.
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	Controls were used, and assume it was just a lake water control given the study says "no solvent carriers were used and all toxicants were tested at concentrations below their reported solubility."
Metric 5:	Negative Control Response	Low	× 1	3	No response was reported for control.
Metric 6:	Randomized Allocation	High	× 1	1	Concentrations were distributed randomly between the two diluters, and for the acute test it was not mentioned whether animals were distributed randomly amongst control groups, but study did report that procedures followed standard American Society for Testing and Materials experimental procedure and as described previously. For the chronic test, embryos were added five at a time into 56 different egg cups which then randomly were added to the treatment and control chambers so that each tank received one cup.
Domain 3: Exposure Characterization					

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Study Citation: Broderius, S. J., Kahl, M. D., Elonen, G. E., Hammermeister, D. E., Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Chronic (>21 days); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	Flow through test was conducted, which is better for volatile chemicals like TCE. It was not specifically noted whether the containers were covered and how much headspace was allowed, but the flow through nature of the test should help with keeping TCE conc consistent. Also measured concentrations were taken.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	Continuous flow through with a check for any spikes. See following description "To verify the accuracy of the method of analyses, known amounts of toxicant were added to control water so that toxicant concentrations could be corrected routinely for spiked water recoveries. At least one spike sample was prepared for each sample set. A mean percent recovery of 90 to 110 percent usually was observed. All statistical/model analyses were based on measured concentrations."
Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	"Methods of chemical analysis included high-pressure liquid chromatography with ultraviolet or diode array/fluorescence detectors, and gasliquid chromatography with flame ionization or electron capture detectors. Samples of all test solutions were taken from the test chambers and toxicant mixing trays and analyzed according to a monitoring program that characterized the toxicant exposures. Duplicate measurements were made routinely with each analytical series to define the reproducibility (precision) of the measurements. Percentage duplicate agreements usually were greater than 90 percent. To verify the accuracy of the method of analyses, known amounts of toxicant were added to control water so that toxicant concentrations could be corrected routinely for spiked water recoveries. At least one spike sample was prepared for each sample set. A mean percent recovery of 90 to 110 percent usually was observed. All statistical/model analyses were based on measured concentrations."
Metric 10:	Exposure Duration and Frequency	High	× 1	1	Continuous exposure for 32 days which is one recommended option for the fish acute guidelines from OECD 210 for fathead minnows

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Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Chronic (>21 days); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	5 exposure groups. at least 5 is recommended by OECD 210 guidelines
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	TCE concentrations tested were below TCE's solubility.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	Fathead minnow are an acceptable test species according to OECD 210 guidelines.
	Metric 14: Acclimatization and Pretreatment Conditions	High	× 1	1	Acclimatization took place, and fish were cultured in the lab with appropriate temp for fathead minnow, and ph of 7.8 was within the range of OECD 210 guidelines. Water hardness was also in the range of OECD 210 guidelines.
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	80 embryos are recommended by OECD 210. This study did " . . . 40 embryos were added to each of the replicated treatment levels and controls." There were at least two replicates.
	Metric 16: Adequacy of Test Conditions	Medium	× 1	2	Housing for the most part are acceptable, but test water was obtained from lake superior and underwent sand filtration. There could be other contaminants in the water, however because controls were used, and chemicals measurement and analysis took place, only minor uncertainties regarding this detail. The appropriate temp for fathead minnow was used, and the ph of 7.8 within the range of OECD 210 guidelines. Water hardness was also in the range of OECD 210 guidelines.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	EC50 and EC20 were measured and determined for growth for chronic toxicity.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was consistent between study groups.
Domain 6: Confounding / Variable Control					

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Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
Data Type: Chronic (>21 days); Aquatic; Fish  
Hero ID: 3665276

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences in study groups from environmental conditions of other factors that could influence the outcome of assessment.
	Metric 20: Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes were not reported for each study group, but this is unlikely to have substantial impacts on results.

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Domain 7: Data Presentation and Analysis

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Study Citation: Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow (*Pimphales promelas*). 24:3117-3127  
 Data Type: Chronic (>21 days); Aquatic; Fish  
 Hero ID: 3665276

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Metric 21:	Statistical Methods	Medium	× 1	2	Only minor uncertainties in the method used to derive the EC values. The study says, "Growth (final wet wt) was selected as the graded sublethal response for the ELS studies because it represents an integrated activity of the whole organism. For the ELS tests, growth was defined as the wet weight of a fathead minnow at the end of a 32-d experiment. The concentrations that reduce the growth by 50 or 20 percent compared to the controls ( <i>i.e.</i> , EC50 and EC20 values) were calculated from a toxicity relationship analysis program developed by Erickson [34]. With this program, the effects of toxicants on wet weight of fathead minnows from early life-stage toxicity tests were analyzed by weighted leastsquares nonlinear regression analysis of the dependant variable ( <i>i.e.</i> , wet wt) versus the independent variable <i>x</i> ( <i>i.e.</i> , toxicant concentration). Three mathematical forms of the regression curves that were used are the logistic equation, piecewiselinear, and piecewise-tailed regression. The regression using the logistic equation produces a sigmoid-shaped curve with infinite tails, a form similar to probit analysis. Piecewise linear regression produces an effects curve in which the effects variable is constant at a control value below a threshold exposure (EC0) and declines linearly above this threshold until it reaches a value of zero at some finite exposure (EC100). The piecewise tailed regression provides a curve that is sigmoid shaped like the logistic equation, but has finite tails and, thus, a finite EC0 and EC100 like the piecewise linear regression. The wet weight of fathead minnows that survived from each individual ELS toxicity test was analyzed by these three regression models. The model that provided the best visual and statistical fit to the experimental data was used to define the test results. Usually that was the piecewise linear or piecewise tailed regression model. A logarithm exposure variable transformation was used in all regression analyses to improve error homogeneity in regression analyses. The analysis provides final estimates for the regression parameters, their standard errors, and 95 percent confidence limits of the best estimate of mean effects. These EC50 estimates and confidence limits then were illustrated graphically."

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Study Citation:	Broderius, S. J.,Kahl, M. D.,Elonen, G. E.,Hammermeister, D. E.,Hoglund, M. D.. 2005. A Comparison of the Lethal and Sublethal Toxicity of Organic Chemical Mixtures to the Fathead Minnow ( <i>Pimphales promelas</i> ). 24:3117-3127				
Data Type:	Chronic (>21 days); Aquatic; Fish				
Hero ID:	3665276				
Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
	Metric 22: Reporting of Data	Medium	× 2	4	Data were not reported for outcomes with negative findings, but this is unlikely to have a substantial impact on results.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes reported.
Overall Quality Determination <sup>‡</sup>		High		1.4	
Extracted		Yes			

\* MWF = Metric Weighting Factor

<sup>†</sup> High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

<sup>‡</sup> The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left[ \sum_i (\text{Metric Score}_i \times \text{MWF}_i) / \sum_j \text{MWF}_j \right]_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

<sup>††</sup> Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.



Study Citation: Leblanc, G. A., Surprenant, D. C.. 1980. The Chronic Toxicity of 8 of the 65 Priority Pollutants to the Water Flea (*Daphnia magna*).  
 Data Type: Chronic (>21 days); Aquatic; Invertebrates  
 Hero ID: 3683110

Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Domain 1: Test Substance					
Metric 1:	Test Substance Identity	High	× 2	2	Trichloroethylene identified by name
Metric 2:	Test Substance Source	High	× 1	1	Aldrich chemical co
Metric 3:	Test Substance Purity	High	× 1	1	98 percent purity - lot number 041557
Domain 2: Test Design					
Metric 4:	Negative Controls	Medium	× 2	4	a solvent and negative control were used, but it did not say what the solvent was.
Metric 5:	Negative Control Response	Unacceptable	× 1	4	Biological response of negative control and solvent control was reported, but there was only 50 percent and 57 percent survival of negative and solvent control. There fore the results of this study are unacceptable.
Metric 6:	Randomized Allocation	High	× 1	1	
Domain 3: Exposure Characterization					
Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	test was conducted in a closed system. Dissolved O, temperature, and pH were measured throughout the experiment. However despite efforts to produce a closed system, volatilization of other volatile chemicals did occur. Because TCE is a volatile chemical other studies have shown rapid volatilization of the chemical from test media within 24 and 48 hours.
Metric 8:	Consistency of Exposure Administration	High	× 1	1	No differences were reported.
Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	While it was attempted to measure TE concentrations authors were unable to reliably measure the chemical and therefore relied on nominal concentrations for this chronic test.
Metric 10:	Exposure Duration and Frequency	Low	× 1	3	duration is good at 21 days which is recommended in OECD 211, however it is unclear how often the test conc is renewed, which could have substantial results on the experiment given that this chemical is highly volatile.

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Study Citation: Leblanc, G. A., Surprenant, D. C.. 1980. The Chronic Toxicity of 8 of the 65 Priority Pollutants to the Water Flea ( <i>Daphnia magna</i> ).					
Data Type: Chronic (>21 days); Aquatic; Invertebrates					
Hero ID: 3683110					
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Daphnids were exposed to five concentrations of each compound, and concentrations were determined by doing an acute range finding test.
	Metric 12: Testing at or Below Solubility Limit	High	× 1	1	conc were below solubility level.
Domain 4: Test Organism					
	Metric 13: Test Organism Characteristics	High	× 2	2	daphnia magna
	Metric 14: Acclimitization and Pretreatment Conditions	N/A		N/A	
	Metric 15: Number of Organisms and Replicates per Group	High	× 1	1	10 daphnia per container and done in triplicate
	Metric 16: Adequacy of Test Conditions	Unacceptable	× 1	4	it appears the closed nature and no cleaning of the system during the experiment produced bacteria and algae growth that affected the health of the daphnia in both controls between day 15 and 21.
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	High	× 2	2	no reported differences among the study groups in environmental conditions or other factors that could influence the outcome of the assessment.
	Metric 20: Outcomes Unrelated to Exposure	Unacceptable	× 1	4	controls had very low survival rate of 50 percent and 57 percent
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	N/A		N/A	not applicable because a chronic toxicity endpoint was not determined
	Metric 22: Reporting of Data	High	× 2	2	results were reported for each exposure group
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	unexpected outcomes were discussed, <i>e.g.</i> , the high rate of mortality in controls and the growth of bacteria and algae.
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Study Citation: Leblanc, G. A., Surprenant, D. C.. 1980. The Chronic Toxicity of 8 of the 65 Priority Pollutants to the Water Flea (*Daphnia magna*).  
 Data Type: Chronic (>21 days); Aquatic; Invertebrates  
 Hero ID: 3683110

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Domain	Metric	Rating <sup>†</sup>	MWF*	Score	Comments <sup>††</sup>
Overall Quality Determination <sup>‡</sup>		Unacceptable		4.0	
Extracted		No			

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\*\* Consistent with our *Application of Systematic Review in TSCARisk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, three of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

\* MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lceil \frac{\sum_i (\text{Metric Score}_i \times \text{MWF}_i)}{\sum_j \text{MWF}_j} \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases}$$

where High:  $\geq 1$  to  $< 1.7$ ; Medium:  $\geq 1.7$  to  $< 2.3$ ; Low:  $\geq 2.3$  to  $\leq 3$ . If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† Metrics that are rated 'High' met the criteria for high confidence as expected for this type of study, and may not require additional comments.