United States
Environmental Protection Agency

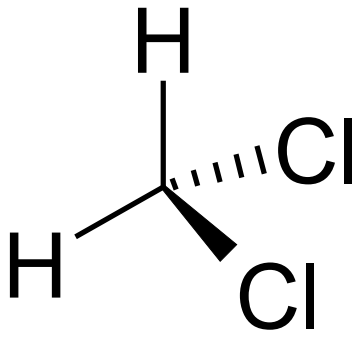
Office of Chemical Safety and
Pollution Prevention

Risk Evaluation for Methylene Chloride

Systematic Review Supplemental File:

Data Quality Evaluation of Environmental Hazard Studies

CASRN: 75-09-2



June 2020

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93660	Acute (0-96 hour); Aquatic; Fish	Black, J. A.,Birge, W. J.,McDonnell, W. E.,Westerman, A. G.,Ramey, B. A.,Bruser, D. M.. 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. 133	**27**
200570	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 Artemia salina larvae to seven chlorinated solvents. Bulletin of Environmental Contamination and Toxicology 59:445-451	**30**
661061	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	**33**
1042080	Acute (0-96 hour); Aquatic; other Amphibians	Marquis, O.,Millery, A.,Guittonneau, S.,Miaud, C.. 2006. Solvent toxicity to amphibian embryos and larvae. Chemosphere 63:889-892	**36**
1486051	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	**38**
2803221	Acute (0-96 hour); Aquatic; other fathead minnow cell-line; total protein content	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	**41**
3493045	Acute (0-96 hour); Aquatic; other Algae; MDA	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31	**44**
3493045	Acute (0-96 hour); Aquatic; other Algae; Chlorophyl a	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31	**47**
3493045	Acute (0-96 hour); Aquatic; other Algae; Protein Content	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31	**49**
3493045	Acute (0-96 hour); Aquatic; other Algae; CAT and SOD	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31	**52**
3493045	Acute (0-96 hour); Aquatic; other Algae; Growth	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31	**55**
3559784	Acute (0-96 hour); Aquatic; other soil fungi	Steiman, R.,Seiglemurandi, F.,Guiraud, P.,Benoitguyod, J. L.. 1995. TESTING OF CHLORINATED SOLVENTS ON MICROFUNGI. Environmental Toxicology and Water Quality 10:283-285	**58**

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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane
	Metric 2:	Test Substance Source	Medium	× 1	2	Indicated from commercial sources, but source never identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Indicated > = 80 percent purity for all chemicals tested; but not specific for dichlomethane.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	A diluent water negative control was used.
	Metric 5:	Negative Control Response	High	× 1	1	Less than 10 percent morality in control populations.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Random allocation indicated, but method used for randomization is unclear.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Indicated 15 daphnids in one test vessel covered with plastic wrap to reduce risk of loss of substance via volatilization, but did not reduce headspace so some volatilization expected.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Distilled water was used to prepare stock solutions of water soluble test substances.
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations, attempt to reduce loss of substance via volatilization but uncertain whether sufficient measures to reduce loss.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Test duration of 48-hours was adequate for determining effects on Daphnia magna.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Indicated 5-8 nominal concentrations, but number of concentrations specific for methylene chloride and spacing of test concentrations is unclear.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test results are below the water solubility of methylene chloride.
Domain 4: Test Organism						

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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†	MWF*	Score	Comments††
	Metric 13:	Test Organism Characteristics	High	× 2	2	Daphnia magna are well-known aquatic invertebrates and suitable for freshwater toxicity tests.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Did not indicate pretreatment conditions, but referred to use of procedures from "methods for acute toxicity tests with fish, macroinvertebrates and amphibians (U.S. EPA 1975).
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	There were 15 daphnia per test concentration and 3 replicates tested.
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	Referred to procedures from "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians (U.S. EPA 1975)."
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Daphnia mortality was recorded at 24 hours and 48 hours, and water quality characteristics (pH, dissolved oxygen, and temperature) were measured at test initiation and termination.
	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	Water quality parameters appeared to be within acceptable limits for daphnia magna as characterized by OECD Test Guideline 202.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	Author appeared to use the moving average angle method with 95 percent confidence limits, based on nominal concentrations, to calculate the LC50 for methylene chloride, but the data to reproduce these result were not provided.
	Metric 22:	Reporting of Data	High	× 2	2	
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	Control mortality was less than 10 percent.
Overall Quality Determination‡			High		1.5	

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

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	The study indicates that all chemicals tested were purchased from commercial chemical suppliers, but does not specify what company.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Study reports a minimum purity of 80 percent for all chemicals tested, but does not specify what the purity is for DCM.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Low	× 1	3	Control mortality is mentioned, but no further details.
	Metric 6:	Randomized Allocation	High	× 1	1	
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Did not cap DCM as with other volatile chemicals tested.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Nominal concentrations were used and were not measured. DCM is volatile.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Study used EPA's "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" which requires that static tests have 10 organisms/treatment divided into >= two test chambers. Test concentrations and spacing were not specified.
	Metric 12:	Testing at or Below Solubility Limit	Low	× 1	3	Test substance concentration was not reported, and it was reported that for some concentrations there was undissolved chemical.
Domain 4: Test Organism						

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	18064

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Test animals utilized were young of the year bluegill (L. macrochirus) obtained from commercial fish suppliers within the continental United States.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Fish were observed for 48 hours and not used if had >3 percent mortality, but acclimation time (i.e. 12 days in the lab) was not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	× 1	2	There are minor uncertainties around number of organisms used.
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	There are minor uncertainties around housing conditions (ex. headspace in jar, DO concs).
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	Low	× 2	6	The study did not provide enough information to allow a comparison of environmental conditions
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	The study did not provide enough information about health outcomes of each study group.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	The method used to calculate LC50s for DCM is unclear: Harris method (Harris, 1959) or the log probit method, a modification of the Litchfield and Wilcox (1949) method.
	Metric 22:	Reporting of Data	Low	× 2	6	Exposure-related behavioral effects not reported, only mortality, and effects at each test concentration (e.g. percent mortality at lowest through highest concentration tested) not provided, including controls.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	18064

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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, 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Heitmuller, P. T.,Hollister, T. A.,Parrish, P. R.. 1981. Acute toxicity of 54 industrial chemicals to sheepshead minnows (Cyprinodon variegatus). Bulletin of Environmental Contamination and Toxicology 27:596-604
Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	18110

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	Medium	× 1	2	Unspecified commercial source.
	Metric 3:	Test Substance Purity	Medium	× 1	2	>=80 percent purity, but not specified for DCM.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Medium	× 1	2	Indicated test not acceptable if control mortality exceeded 10 percent but no specific details for DCM test.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomized allocation not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Static system, did not take measures to control volatilization of methylene chloride.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures consistent across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	No analytical monitoring; Nominal concentrations used and methylene chloride is volatile.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Test concentrations determined after range-finding test were not specified.
	Metric 12:	Testing at or Below Solubility Limit	Low	× 1	3	Not specified so uncertain.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Total holding period not indicated.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	housing prior to testing not clear but flowing seawater and feeding conditions indicated.

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	18110

Domain	Metric		Rating†	MWF⋆	Score	Comments††
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	Medium	× 2	4	Uncertain due to minimal details provided for test setup and test methods for this multichemical testing study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Minimal details provided - no health outcomes not related to exposures indicated, but assumed that multichemical test will report significant health issues/mortality.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	Moving average angle analysis, probit analysis, or binomial probability used, but not clear which for DCM.
	Metric 22:	Reporting of Data	Medium	× 2	4	Exposure-related behavioral effects not reported, only mortality, and effects at each test concentration (e.g. percent mortality at lowest through highest concentration tested) not provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Study Citation:	Samoiloff, M.R., Schulz, S., Jordan, Y., Denich, K., Arnott, E.. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode Panagrellus redivivus. Can. J. Fish. Aquat. Sci. 37:1167-1174
Data Type:	Other; Aquatic; Invertebrates
Hero ID:	29147

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	Medium	× 1	2	Two potential source companies indicated, but not specific for methylene chloride.
	Metric 3:	Test Substance Purity	Low	× 1	3	Source company indicated, suggests at least a technical grade; but no purity given.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Low	× 1	3	Negative control responses not provided.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomized allocation not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	DCM's volatility was not addressed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	N/A		N/A	Testing conducted in a medium that would interfere with analytical instruments. Effects were observed at low concentrations suggesting DCM was maintained in the medium.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Unacceptable	× 2	8	The nematode is not a well-known laboratory species, and is not representative of aquatic invertebrates.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	

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Data Type:	Other; Aquatic; Invertebrates
Hero ID:	29147

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	No details on biomass loading, temperature, etc were provided.
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	Low	× 2	6	Study did not provide information on individual environmental conditions for each chemical tested to allow a comparison.
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	Not indicated.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	
	Metric 22:	Reporting of Data	Medium	× 2	4	Control results not provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	29147

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	Medium	× 1	2	Two source companies indicated, but not specific for methylene chloride.
	Metric 3:	Test Substance Purity	Low	× 1	3	Source company indicated, suggests at least a technical grade; but no purity given.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Low	× 1	3	Results reported relative to controls in the developmental test, but negative control results not provided.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomized allocation not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	DCM's volatility was not addressed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	N/A		N/A	Testing conducted in a medium that would interfere with analytical instruments. Effects were observed at low concentrations suggesting DCM was maintained in the medium.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Unacceptable	× 2	8	The nematode is not a well-known laboratory species, and is also not a freshwater species, lives in beer mats; therefore, not relevant for aquatic toxicity.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	29147

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	No details on biomass loading, temperature, etc were provided.
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	Low	× 2	6	Study did not provide information on individual environmental conditions for each chemical tested to allow a comparison.
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	Control health outcomes not indicated.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	
	Metric 22:	Reporting of Data	Medium	× 2	4	Control results not provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Geiger, D. L.,Poirier, S. H.,Brooke, L. T.,Call, D. J. eds. 1986. Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas): volume III.
Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	32170

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source was Aldrich Chemical Company.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was 99+ percent for dichloromethane.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Lake Superior water or municipal city water used for controls.
	Metric 5:	Negative Control Response	Low	× 1	3	Control behavioral effects and mortality data were reported.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Random distribution of test fish indicated but randomization method not described.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Authors noted high volatility of methylene chloride but did not cap test vessels.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Flow-through exposures were made with various systems described by the authors, but exposure system for dichloromethane was not specified.
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Gas-liquid chromatography was used to measure dichloromethane daily in test vessels.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	The tests were flow-through exposures for 96-hours, which is adequate for detection of effects and mortality in fish.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Five exposure concentrations and the control, ranging from 0 to 1120 mg/L nominally, were adequately spaced to develop a concentration-response curve.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Testing was below the water solubility limit for dichloromethane.
Domain 4: Test Organism						

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Study Citation: Geiger, D. L.,Poirier, S. H.,Brooke, L. T.,Call, D. J. eds. 1986. Acute toxicities of organic chemicals to fathead minnows (Pimephales promelas): volume III.
Data Type: Acute (0-96 hour); Aquatic; Fish
Hero ID: 32170

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Organism Characteristics	High	× 2	2	The test organism is a well-known species, Pimephale promelas (fathed minnow), cultured in U.S. EPA lab or at U. of Wisconsin.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Acclimatization and pretreatment conditions were similar to test conditions, minus feeding 24 hours prior to and during testing.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	For the single cell, electronic, or mini exposure systems, 5, 10, or 20 fish per treatment and controls were used, respectively.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	Loading rate was less than 0.5 g/L/day per test vessel and environmental conditions were monitored and similar to pre-test conditions.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	For dichloromethane, dead fish were noted and removed daily, and fish behavior was noted using detailed criteria. The authors used the corrected average of tank test concentrations in analysis.
	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	Environmental conditions were consistent across test groups for dichloromethane. There were no behavioral effects or mortalities reported in the controls.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	There were no behavioral effects or morality reported in controls.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	The authors used Trimmed Spearman-Karber method and corrected average of tank concentrations to assess EC50s and LC50s with 95 percent confidence intervals.
	Metric 22:	Reporting of Data	High	× 2	2	
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.3	

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	32170

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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.

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

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†† 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; other Fish; Static Test
Hero ID:	58126

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as methylene chlorid
	Metric 2:	Test Substance Source	Medium	× 1	2	Substance source not identified, however, the authors work for chemical company that produces methylene chloride.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not indicated.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	A negative control with dechlorinated lake water was used.
	Metric 5:	Negative Control Response	Low	× 1	3	A negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization was not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Test chambers were covered with plastic wrap, but no indication that headspace minimized to reduce volatilization of the test substance.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations were used in the static test, and some loss of methylene chloride is expected from vaporization into the headspace of the plastic covered vessels.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	This was a 96-hour test adequate for determining the median lethal concentration (LC50) in fish.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Test concentrations and spacing of exposure concentrations was not provided.
	Metric 12:	Testing at or Below Solubility Limit	Medium	× 1	2	Test concentrations were not provided, but test results were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Low	× 2	6	Test organisms (fathead minnow) were obtained from a bait company, not cultured for laboratory testing.

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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; other Fish; Static Test
Hero ID:	58126

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Test organisms from a bait company which leaves uncertainty regarding disease, age, and origin of the fish; however, control and test outcomes do not appear to be impacted.
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	Number of organisms and replicates per exposure concentration were not indicated.
	Metric 16:	Adequacy of Test Conditions	Low	× 1	3	Details on test conditions for the static test were not provided.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Dead or affected fish were counted and dead fish were removed from test vessels on a daily basis.
	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	No details regarding the environmental conditions for each exposure concentration or the controls were provided.
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	No information on outcomes unrelated to exposures was provided.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Probit analyis was used to obtain LC10, LC50 and LC90 with 95 percent confidence limits.
	Metric 22:	Reporting of Data	Medium	× 2	4	Results for Flow-through tests were compared to static, but control responses not provided.
	Metric 23:	Explanation of Unexpected Outcomes	Low	× 1	3	No details on unexpected outcomes were provided.
Overall Quality Determination‡			Medium		2.2	
Extracted			Yes			

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Data Type:	Acute (0-96 hour); Aquatic; other Fish; Static Test
Hero ID:	58126

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
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$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

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Data Type:	Acute (0-96 hour); Aquatic; other Fish; Flow-Through Test
Hero ID:	58126

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as methylene chloride.
	Metric 2:	Test Substance Source	Medium	× 1	2	Substance source not identified, however authors work for chemical company that produces methylene chloride.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not indicated.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Controls were used (lake water).
	Metric 5:	Negative Control Response	Low	× 1	3	A negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not indicated.
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	Medium	× 2	4	Gas chromatography used to analyze test concentrations daily for the flow-through test, but few details about methodology provided.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	This 96-hour test was adequate duration for determining the median lethal concentration in fish.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Test concentrations and spacing of exposure concentrations was not provided.
	Metric 12:	Testing at or Below Solubility Limit	Medium	× 1	2	Test concentrations were not provided, but test results were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Low	× 2	6	Test organisms were obtained from a bait company, not cultured for laboratory testing.

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Data Type:	Acute (0-96 hour); Aquatic; other Fish; Flow-Through Test
Hero ID:	58126

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Test organisms were obtained from a bait company, which leaves uncertainty regarding origin (purity and consistency of stock), age, and disease of the fish; however, control results appeared normal so this does not appear to impact test results.
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	The number of organisms and replicates per test concentration was not reported.
	Metric 16:	Adequacy of Test Conditions	Low	× 1	3	Although water quality parameters (pH, D.O, tempurature, etc.) were provided prior to test initiation, details on test conditions (biomass loading, aquaria size, water quality during and after testing) were not provided.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Dead or affected fish were counted and dead fish were removed from test vessels each day in order to assess the median lethal concentration.
	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	No details regarding the environmental conditions during testing were provided.
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	No details on outcomes unrelated to exposures were provided.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Probit analysis was used to obtain LC10, LC50, and LC90 with 95 percent confidence limits.
	Metric 22:	Reporting of Data	High	× 2	2	
	Metric 23:	Explanation of Unexpected Outcomes	Low	× 1	3	No details on unexpected outcomes provided.
Overall Quality Determination‡			Medium		2.1	
Extracted			Yes			

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Data Type:	Acute (0-96 hour); Aquatic; other Fish; Flow-Through Test
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Domain	Metric	Rating†	MWF⋆	Score	Comments††

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Kuhn, R.,Pattard, M.,Pernak, K. D.,Winter, A.. 1989. Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to Daphnia magna. Water Research 23:495-499
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	85242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	Low	× 1	3	Multi-chemical test, no source indicated.
	Metric 3:	Test Substance Purity	Low	× 1	3	No purity or grade information provided.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Medium	× 1	2	Control response not reported, but only used controls with <10 percent inhibition.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Medium	× 2	4	Closed vessel with ground-glass stoppers, but did not reduce headspace to further reduce volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Nominal concentrations for a volatile substance and no analytical monitoring. Headspace in stoppered vessels will still have volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	Specific concentrations not provided for methylene chloride, but range tested to provide 3-4 EC values from EC0 to EC100 and EC50.
	Metric 12:	Testing at or Below Solubility Limit	Low	× 1	3	Specific concentrations not provided for methylene chloride to determine this.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	85242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
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	Medium	× 2	4	Study did not provide environmental information for comparison, but authors indicated that DO and pH were evaluated at the end and factored into the results.
	Metric 20:	Outcomes Unrelated to Exposure	Low	× 1	3	Health outcomes for controls not reported, but authors indicated that controsl with >10 percent inhibition were not used.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	
	Metric 22:	Reporting of Data	Medium	× 2	4	Endpoint values and only use of controls with <10 percent inhibition were reported, but no other details provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			~~Medium~~ ⟶ Low		1.7	Although vessels were capped, headspace allows for volatilization and less certainty of actual methylene chloride in test solution.
Extracted			Yes			

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where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Black, J. A.,Birge, W. J.,McDonnell, W. E.,Westerman, A. G.,Ramey, B. A.,Bruser, D. M.. 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. 133
Data Type:	Acute (0-96 hour); Aquatic; other Amphibians
Hero ID:	93660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	Substance source was not indicated.
	Metric 3:	Test Substance Purity	High	× 1	1	All test substances were reagent grade.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Fish and amphibian control eggs were used.
	Metric 5:	Negative Control Response	High	× 1	1	Control survival ranged from 84-99 percent.
	Metric 6:	Randomized Allocation	Low	× 1	3	There was no mention of randomized allocation of test organisms.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Flow-through testing with closed vessel devoid of air space was used to minimize volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Gas-liquid chromatography was used to measure test concentrations daily.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Amphibian embryo-larvae were exposed up to 4 days post-hatch, sufficient to determine effects in embryos and larvae.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 exposure concentrations with appropriate spacing used fore each amphibian tested.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	All exposure concentrations were below the water solubility of methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Amphibians used were appropriate for this study, with the exception of the African Clawed frog, which is not endemic to the U.S.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Freshly fertilized eggs from amphibians were either obtained from laboratory suppliers or from amphibians cultured in the lab.

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Study Citation: Black, J. A.,Birge, W. J.,McDonnell, W. E.,Westerman, A. G.,Ramey, B. A.,Bruser, D. M.. 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. 133
Data Type: Acute (0-96 hour); Aquatic; other Amphibians
Hero ID: 93660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Single replicates of 50 to 125 eggs were used per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	A loading rat of up to 125 eggs per test concentration was used, which did not appear to impact test results. Environmental conditions were within acceptable ranges, and control mortality was acceptable.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Test vessels observed daily to assess development and remove dead test organisms.
	Metric 18:	Consistency of Outcome Assessment	Medium	× 1	2	LC50, LC10, LC1s were assessed adjusted for control mortality, but detailed control mortality data were not provided.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	Environmental conditions appeared consistent across test concentrations and control mortality ranged from 1 - 16 percent.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Teratogenesis was reportedly infrequent in controls (percent teratogenicity not reported) and control mortality ranged from 1 to 16 percent, which is acceptable.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	Survivability was reported as percent of total organisms at each exposure concentration after corrected for control mortality, but detailed control data were not reported. LC50s, LC10s, and LC1s were calculated using log-probit analysis.
	Metric 22:	Reporting of Data	Medium	× 2	4	All dose-response and survivability data were reported after correction for control mortality, but control data was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.3	

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Data Type:	Acute (0-96 hour); Aquatic; other Amphibians
Hero ID:	93660

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	93660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	Substance source was not indicated.
	Metric 3:	Test Substance Purity	High	× 1	1	All test substances were reagent grade.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Fish and amphibian control eggs were used.
	Metric 5:	Negative Control Response	High	× 1	1	Control survival ranged from 84-99 percent.
	Metric 6:	Randomized Allocation	Low	× 1	3	There was no mention of randomized allocation of test organisms.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Flow-through testing with closed vessel devoid of air space was used to minimize volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Gas-liquid chromatography was used to measure test concentrations daily.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Fish embryo-larvae were exposed up to 4 days post-hatch , sufficient to determine effects in embryos and larvae.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 exposure concentrations with appropriate spacing used for each fish tested.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	All exposure concentrations were below the water solubility of methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Rainbow trout and fathead minnow are well known species. The trout were obtained from a hatchery and freshly fertilized fathead minnow eggs were obtained from the EPA Newtown Fish Toxicology Laboratory.

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Study Citation: Black, J. A.,Birge, W. J.,McDonnell, W. E.,Westerman, A. G.,Ramey, B. A.,Bruser, D. M.. 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. 133
Data Type: Acute (0-96 hour); Aquatic; Fish
Hero ID: 93660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Freshly fertilized eggs from fish were either obtained from laboratory suppliers or from fish cultured in the lab.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Single replicates of 50 to 125 eggs were used per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	A loading rat of up to 125 eggs per test concentration was used, which did not appear to impact test results. Environmental conditions were within acceptable ranges, and control mortality was acceptable.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Test vessels observed daily to assess development and remove dead test organisms.
	Metric 18:	Consistency of Outcome Assessment	Medium	× 1	2	LC50, LC10, LC1s were assessed adjusted for control mortality, but detailed control mortality data were not provided.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	Environmental conditions appeared consistent across test concentrations and control mortality ranged from 1 - 16 percent.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Teratogenesis was reportedly infrequent in controls (percent teratogenicity not reported) and control mortality ranged from 1 to 16 percent, which is acceptable.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	Survivability was reported as percent of total organisms at each exposure concentration after corrected for control mortality, but detailed control data were not reported. LC50s, LC10s, and LC1s were calculated using log-probit analysis.
	Metric 22:	Reporting of Data	Medium	× 2	4	All dose-response and survivability data were reported after correction for control mortality, but control data was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	

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Study Citation:	Black, J. A.,Birge, W. J.,McDonnell, W. E.,Westerman, A. G.,Ramey, B. A.,Bruser, D. M.. 1982. The aquatic toxicity of organic compounds to embryo-larval stages of fish and amphibians. 133
Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	93660

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Overall Quality Determination‡		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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	High	× 1	1	
	Metric 3:	Test Substance Purity	High	× 1	1	Analytical grade
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	Medium	× 1	2	Control response not reported but not expected to affect results. Typically multi-chemical tests will only report control results if significant (i.e. > 10 percent mortality)
	Metric 6:	Randomized Allocation	Medium	× 1	2	Randomized allocation not explicitly stated, but method of allocation of organisms to study groups implies randomized selection: "For toxicity testing, samples of 10 larvae each were added to 1 mL of syntheticseawater in plastic 16-mm petri dishes containing..."
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Nominal concentrations used without steps to reduce volatilization of methylene chloride.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	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 methylene chloride.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	24-72 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 methylene chloride. LC50/ EC50s were determined indicating exposure concentrations sufficiently spaced.

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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†	MWF⋆	Score	Comments††
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	10 animals and four replicates per methylene chloride concentration tested.
	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	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	Medium	× 2	4	Control results not provided, but unlikely to impact results.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	

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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†	MWF⋆	Score	Comments††
Overall Quality Determination‡		~~High~~ ⟶ Low		1.5	Nominal concentrations without analytical measurement or measures to reduce volatilization of methylene chloride during testing.
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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Promochem was the source company for methylene choride.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported as nanograde.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Indicated negative controls were used.
	Metric 5:	Negative Control Response	High	× 1	1	Exponential growth was reported in control algae.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Headspace was removed in sealed test vials to minimize volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Measurments by Gas chromatography/ECD after liquid-liquid microextraction were taken at test initiation and end.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Algae were treated for 72 hours under static conditions.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Seven treatment groups plus controls (positive and negative) were tested. Test concentrations were not reported, but are shown in the figures, which show adequate spacing of the test concentrations to determine concentration response curves for methylene chloride.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Chlamydomonas reinhardtii is a mobile algal species with two flagella, not commonly used for toxicity testing.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	

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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†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Two replicates per test concentration and 3 negative controls were reported with an initial inoculum of 5 x $10\hat{3}$ cells/mL each. OECD test guideline recommends 3 replicates unless a NOEC is not required, which was the case here.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	Culture conditions appear adequate and negative control results showed uninhibited exponential growth.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Biomass assessed using fluorometric measurement of total chlorophyll for controls and treatment groups to determined EC10s and EC50s.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	No inconsistencies were reported, and both positive and negative controls performed as expected.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	Both positive and negative controls performed as expected, differences in environmental conditions were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	Positive and negative controls performed as expected and no outcomes unrelated to exposures were reported.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Probit analysis was used to assess significant differences in biomass.
	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.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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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†	MWF⋆	Score	Comments††

⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Marquis, O.,Millery, A.,Guittonneau, S.,Miaud, C.. 2006. Solvent toxicity to amphibian embryos and larvae. Chemosphere 63:889-892
Data Type:	Acute (0-96 hour); Aquatic; other Amphibians
Hero ID:	1042080

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	Low	× 1	3	Source not identified
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not identified
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	
	Metric 5:	Negative Control Response	High	× 1	1	
	Metric 6:	Randomized Allocation	High	× 1	1	
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Volatility of the test material was not taken into account. The test concentrations were not measured and endpoints reported in terms of nominal concentration.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Exposure concentrations were not measured
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Not all exposure groups were specified
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Embryos were collected in the wild from agricultural landscape, but no additional details about potential exposure to pesticides was discussed. Lack of mortality in controls indicates this wasn't an issue.
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Acclimatization procedure was not discussed.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	20
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	

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Study Citation:	Marquis, O.,Millery, A.,Guittonneau, S.,Miaud, C.. 2006. Solvent toxicity to amphibian embryos and larvae. Chemosphere 63:889-892
Data Type:	Acute (0-96 hour); Aquatic; other Amphibians
Hero ID:	1042080

Domain	Metric		Rating†	MWF⋆	Score	Comments††
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	Low	× 1	3	Significance test methodology was not explained or reported
	Metric 22:	Reporting of Data	Medium	× 2	4	Data summary was presented, whole dataset was not presented
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	The study authors did not identify the test substance source.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was reported as a minimum of 97 percent.
Domain 2: Test Design						
	Metric 4:	Negative Controls	Medium	× 2	4	A negative control was used, but the study authors did not specify the type of control used for the methylene chloride test (i.e. dilution water, solvent control, or filtered water not specified).
	Metric 5:	Negative Control Response	High	× 1	1	The negative control response was less than or equal to 10 percent.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	The authors used teflon-lined screw caps with no air space to minimize test substance loss.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure concentrations were not provided, but all test substances were diluted into a minimum of 5 exposure concentrations plus a control for each test.
	Metric 9:	Measurement of Test Substance Concentration	Medium	× 2	4	Only nominal concentration were used, but measures were taken to limit test chemical loss in the test chambers.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	These were static 48-hour tests which is sufficient for determining acute effects in aquatic invertebrates.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	× 1	2	There were 5 exposure concentrations plus controls for each test substance, but concentrations used and spacing of exposure levels was not reported.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Median 48-hour mortality was observed at concentrations below the water solubility limit.

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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†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Daphnia magna, a well-known laboratory test organism, were used.
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	The study did not report whether organisms were acclimatized.
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	The number of organisms and replicates not indicated.
	Metric 16:	Adequacy of Test Conditions	Low	× 1	3	The temperatures (21-25”C) at which the daphnia were kept were higher than the recommended range of temperatures from OECD Test Guideline 202 (18-22”C) and test temps varied greater than 1C. Loading rate was unclear, but does not appear to have affected results.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Median lethal concentration (LC50s) were reported.
	Metric 18:	Consistency of Outcome Assessment	Medium	× 1	2	Incomplete details of test protocol reported, however, study indicated that results were corrected for control mortality (which is less than 10 percent).
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; however, this is not likely to have significant impact on study results as controls had <10 percent mortality.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition for each study group was not reported, but controls had < 10 percent mortality, so unlikely to have significant impact on results.
Domain 7: Data Presentation and Analysis						

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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†	MWF⋆	Score	Comments††
	Metric 21:	Statistical Methods	Medium	× 1	2	The percent mortality at each concentration was corrected for control mortality(always < 10 percent). Nominal medial lethal concentrations (LC50 values), slope factors, and 95 percent confidence limits were calculated from graphs by using Litchfield and Wilcoxon (1948) methodology.
	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 and or data were only reported for some outcomes.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	No unexpected outcomes were reported.
Overall Quality Determination‡			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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	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; other fathead minnow cell-line; total protein content
Hero ID:	2803221

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	DCM source not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not indicated.
Domain 2: Test Design						
	Metric 4:	Negative Controls	Medium	× 2	4	A control was used, but not much detail is given.
	Metric 5:	Negative Control Response	Low	× 1	3	Negative control response was not reported, in vitro test.
	Metric 6:	Randomized Allocation	Low	× 1	3	Not reported whether cells were allocated randomly-in vitro test.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	DCM was solubilized in paraffin for this 24 hr in-vitro test, but not clear that this reduced loss of test substance from volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	× 1	2	This was a 24-hour static test in fish cells, and effects were observed, but there is uncertainty whether exposure duration was adequate.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Low	× 1	3	Number of exposure groups and spacing were not reported, but an EC50 was calculated.
	Metric 12:	Testing at or Below Solubility Limit	Unacceptable	× 1	4	Figure 1 in the paper indicates that the minimum concentration tested and the EC50 are higher than the water solubility for DCM, and there were no analytical measurements of the test concentrations.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Cultured fathead minnow cells from posterior of anus, unclear whether acceptable for this type of in vitro test.

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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; other fathead minnow cell-line; total protein content
Hero ID:	2803221

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	The number of replicates was not indicated, but there were 6 x $10\hat{5}$ cells/0.6 mL test well. It is unclear whether the cell concentration was adequate for this test.
	Metric 16:	Adequacy of Test Conditions	Low	× 1	3	Control response was not reported, and it is unclear whether test conditions were adequate for this test.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Total protein inhibition (EC50) was used to determine cytotoxicity.
	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 DCM for each test concentration.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	The statistical method used to derive the EC50 was not reported in detail.
	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 did not find a linear correlation between the published LC50 levels and the EC50 values from this test and the reasoning for the discrepancy was unclear.
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			Yes			

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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; other fathead minnow cell-line; total protein content
Hero ID:	2803221

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; MDA
Hero ID:	3493045

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Use of controls was indicated.
	Metric 5:	Negative Control Response	High	× 1	1	Results reported as relative to controls and control responses were shown in the figures for each endpoint measured.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not described.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Test concentrations were not measured and no effort to prevent loss through volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Volatility of the test substance was not taken into account. Test concentrations were not measured to account for potential loss through volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	96-hour exposures prior to MDA assay.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 concentrations tested plus a control, and spacing was adequate for dose-response analysis.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Chlorella vulgaris is a well-known green algae species.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Pretreatment conditions were not described, but this was not expected to have an impact on the results, as evidenced by control performance.

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; MDA
Hero ID:	3493045

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Initial concentration of 8 x 10^{5} cells/mL with 3 replicates per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	N/A		N/A	Referred to Zhang and Kirkham (1994) methodology for analyzing MDA, relative to protein content.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Results were reported relative to controls and protein content.
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	Authors did not report outcomes that affected controls, but did not indicate that control MDA enzymes were within an acceptable range; however, the authors report results relative to control values.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Results were reported as the mean +/- standard error and used ANOVA to test for significance, with less than 0.05 or 0.01 probability.
	Metric 22:	Reporting of Data	High	× 2	2	Regression equation, correlation coefficient and significance reported, along with NOEC, and figures representing treatments relative to controls were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; MDA
Hero ID:	3493045

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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⋆ MWF = Metric Weighting Factor

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Chlorophyl a
Hero ID:	3493045

Domain	Metric		Rating†	MWF*	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material was identified as methylene chloride
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance 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	Use of controls indicated.
	Metric 5:	Negative Control Response	High	× 1	1	Percent inhibition values were reported relative to controls and control responses indicated in figures.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not described.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Test concentrations were not measured and no effort to prevent loss through volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	No analytical measurement of volatile test substance.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	96-hour exposures prior to Chlorophyl a assay.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 concentrations tested plus a control, and spacing was adequate for dose-response analysis.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Chlorella vulgaris is a well-known species of algae.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Pretreatment conditions were not described, but this was not expected to have an impact on the results, as evidenced by control performance.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Initial concentration was 8 x 10^5 cells/mL with 3 replicates per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Chlorophyl a
Hero ID:	3493045

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	N/A		N/A	Referred to Inskeep and Bloom (1985) method for chlorophyll a analysis.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Results were reported relative to controls.
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	Authors did not report outcomes that affected control outcomes, but did not indicate that control results were within acceptable ranges for the test assays. The authors do report results relative to control values.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Results were reported as the mean +/- standard error and used ANOVA to test for significance, with less than 0.05 or 0.01 probability.
	Metric 22:	Reporting of Data	High	× 2	2	Regression equation, correlation coefficient and significance reported, along with EC50.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			Yes			

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

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Protein Content
Hero ID:	3493045

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance 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	Use of controls was indicated.
	Metric 5:	Negative Control Response	High	× 1	1	Results reported as relative to controls and control responses were shown in the figures for each endpoint measured.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not described.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	No effort to prevent loss through volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Test concentrations were not measured and no effort to prevent loss through volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	96-hour exposures prior to protein content assay.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 concentrations tested plus a control, and spacing was adequate for dose-response analysis.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Chlorella vulgaris is a well-studied green algae species.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Pretreatment conditions were not described, but this was not expected to have an impact on the results, as evidenced by control performance.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Initial concentratios were 8 x 10^5 cells/mL with 3 replicates per test concentration.

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Protein Content
Hero ID:	3493045

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 16: Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment					
	Metric 17: Outcome Assessment Methodology	High	× 2	2	Protein content was determined using a BCA protein assay.
	Metric 18: Consistency of Outcome Assessment	High	× 1	1	Results were reported relative to controls.
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	Authors did not report outcomes that affected controls, and did indicate whether control protein content was within an acceptable range; however, the authors reported assay results relative to control values.
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	High	× 1	1	
	Metric 22: Reporting of Data	High	× 2	2	Regression equation, correlation coefficient and significance reported, along with NOEC, and figures representing treatments relative to controls were reported.
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡		Unacceptable		4.0	
Extracted		No			

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Protein Content
Hero ID:	3493045

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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⋆ MWF = Metric Weighting Factor

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where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.

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Data Type:	Acute (0-96 hour); Aquatic; other Algae; CAT and SOD
Hero ID:	3493045

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance 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	Controls were used for the enzyme tests.
	Metric 5:	Negative Control Response	High	× 1	1	Results reported as relative to controls and control responses were shown in the figures for each endpoint measured.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization not described.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Volatility of the test substance was not taken into account.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Volatility of the test substance was not taken into account. Test concentrations were not measured to account for potential loss through volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	96-hour exposures prior to the enzyme assays.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 concentrations tested plus a control, and spacing was adequate for dose-response analysis.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Testing was below the water solubility limit.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Chlorella vulgaris is a well-studied algae species.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Pretreatment conditions were not described, but this was not expected to have an impact on the results, as evidenced by control performance.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Initial concentration of 8 x $10\hat{5}$ cells/mL with 3 replicates per test concentration.

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; CAT and SOD
Hero ID:	3493045

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Catalase activity was assessed as the amount of H2O2 degraded per minute at 25C. and SOD was the amount of enzyme inhibiting 50 percent of a water soluble tetrazolium (indicator of superoxide radicals).
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Results were reported relative to controls.
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	Authors did not report outcomes that affected controls, and did not indicate that control enzymes were within an acceptable range; however, the authors report results relative to control values.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Results were reported as the mean +/- standard error and used ANOVA to test for significance, with less than 0.05 or 0.01 probability.
	Metric 22:	Reporting of Data	High	× 2	2	Regression equation, correlation coefficient and significance reported, along with NOEC, and figures representing treatments relative to controls were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; CAT and SOD
Hero ID:	3493045

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Growth
Hero ID:	3493045

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance 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	A negative control was reported.
	Metric 5:	Negative Control Response	High	× 1	1	Results were reported as relative to controls and control responses were shown in the figures for each endpoint measured.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	Volatility of the test substance was not taken into account. Capping of test vessels and no headspace needed to reduce volatilization of DCM.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Test concentrations were not measured to account for potential loss through volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Algae were exposed to methylene chloride for 96-hours to assess growth inhibition.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 concentrations tested plus a control, and spacing was adequate for dose-response analysis.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	.Chlorella vulgaris is a well-known green algae species.
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	Pretreatment conditions were not described, but this was not expected to have an impact on the results, as evidenced by control performance.

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Data Type:	Acute (0-96 hour); Aquatic; other Algae; Growth
Hero ID:	3493045

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Initial concentration of 8 x 10^{5} cells/mL with 3 replicates per test concentration.
	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	Results were reported relative to controls and protein content.
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	Authors did not report outcomes that affected controls, but did not indicate that control growth rates were within an acceptable range; however, the authors reported growth rate for treatment populations relative to controls.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Results were reported as the mean +/- standard error and used ANOVA to test for significance, with less than 0.05 or 0.01 probability.
	Metric 22:	Reporting of Data	High	× 2	2	Regression equation, correlation coefficient and significance reported, along with EC50 and NOEC, and figures representing treatments relative to controls were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Study Citation:	Wu, S.,Zhang, H.,Yu, X.,Qiu, L.. 2014. Toxicological Responses of Chlorella vulgaris to Dichloromethane and Dichloroethane. Environmental Engineering Science 31
Data Type:	Acute (0-96 hour); Aquatic; other Algae; Growth
Hero ID:	3493045

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Steiman, R.,Seiglemurandi, F.,Guiraud, P.,Benoitguyod, J. L.. 1995. TESTING OF CHLORINATED SOLVENTS ON MICROFUNGI. Environmental Toxicology and Water Quality 10:283-285
Data Type:	Acute (0-96 hour); Aquatic; other soil fungi
Hero ID:	3559784

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane (DCM).
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was from a chemical company.
	Metric 3:	Test Substance Purity	Low	× 1	3	Not indicated
Domain 2: Test Design						
	Metric 4:	Negative Controls	Unacceptable	× 2	8	No control.
	Metric 5:	Negative Control Response	N/A		N/A	No control used.
	Metric 6:	Randomized Allocation	Low	× 1	3	No randomization indicated
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	A closed system was used to reduce volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Medium	× 2	4	Not measured, however, due to differences in volatility, the atmosphericconcentrations were not the same for the different solvents tested: 2.40 g L-1 for DCM .
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A		N/A	Each solvent had one exposure concentration determined by the atmospheric pressure of the test vessel.
	Metric 12:	Testing at or Below Solubility Limit	N/A		N/A	Each solvent had one exposure concentration determined by the atmospheric pressure of the test vessel.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	The sediment fungal strains were cultivated in petri dishes on synthetic solid medium of Galzy and Slonimski (I 957)(glucose 5 g L - 1).
	Metric 15:	Number of Organisms and Replicates per Group	N/A		N/A	Each solvent had one exposure concentration determined by the atmospheric pressure of the test vessel; testing on sediment fungal species.

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Study Citation:	Steiman, R.,Seiglemurandi, F.,Guiraud, P.,Benoitguyod, J. L.. 1995. TESTING OF CHLORINATED SOLVENTS ON MICROFUNGI. Environmental Toxicology and Water Quality 10:283-285
Data Type:	Acute (0-96 hour); Aquatic; other soil fungi
Hero ID:	3559784

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Mortality of all test species increased with time.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	N/A		N/A	No further information was provided to determine if non-treatment related differences between test groups.
	Metric 20:	Outcomes Unrelated to Exposure	N/A		N/A	No further information was provided to determine outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	N/A		N/A	Not provided.
	Metric 22:	Reporting of Data	Low	× 2	6	Results were reported but not raw data.
	Metric 23:	Explanation of Unexpected Outcomes	N/A		N/A	Not provided.
Overall Quality Determination‡			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

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

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Dill, D. C.,Murphy, P. G.,Mayes, M. A.. 1987. TOXICITY OF METHYLENE-CHLORIDE TO LIFE STAGES OF THE FATHEAD MINNOW, PIMEPHALES-PROMELAS RAFINESQUE. Bulletin of Environmental Contamination and Toxicology 39:869-876
Data Type:	Other; Aquatic; Fish
Hero ID:	3587456

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance was obtained from Burdick and Jackson Laboratories, Muskegon, Michigan.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was >99.9 percent.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Negative controls were used in the test.
	Metric 5:	Negative Control Response	High	× 1	1	Negative control response were reported- for larval survival, embryo hatch and normal larvae at hatch.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomization was indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Test vessels were covered to prevent evaporation, but headspace was not addressed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	The test concentrations were reported in terms of nominal test concentrations despite volatility and head space in test vessels.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Flow-through test that lasted 28-days post-hatch.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	There were 6 nominal test concentrations, ranging from 81 mg/L to 433 mg/L of methylene chloride, plus a control, with adequate spacing to detect a concentration-response relationship.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Testing was below the water solubility limit for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Fathead minnow embryos less than 24-hours old were obtained from Dow Chemical Company.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Pretreatment conditions were appropriate for the test.

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Study Citation:	Dill, D. C.,Murphy, P. G.,Mayes, M. A.. 1987. TOXICITY OF METHYLENE-CHLORIDE TO LIFE STAGES OF THE FATHEAD MINNOW, PIMEPHALES-PROMELAS RAFINESQUE. Bulletin of Environmental Contamination and Toxicology 39:869-876
Data Type:	Other; Aquatic; Fish
Hero ID:	3587456

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Four replicates of 15 embryos were exposed to each treatment concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Dead embryos and dead/deformed larvae were counted and removed daily. Hatched embryos, including dead or deformed were also counted daily.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Embryo-larval survival and hatchability assessed for treatment groups and controls.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	There were no outcomes unrelated to exposures reported.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Percent hatched, normal larvae at hatch survival data were normalized with arcsine transformation and compared to mean weights using one-way ANOVA. Dunnett's one-tailed t-test was used to compare treatments to controls.
	Metric 22:	Reporting of Data	High	× 2	2	Data were reported in the results and discussion and in table format. A MATC was calculated for body weight.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	Dill, D. C.,Murphy, P. G.,Mayes, M. A.. 1987. TOXICITY OF METHYLENE-CHLORIDE TO LIFE STAGES OF THE FATHEAD MINNOW, PIMEPHALES-PROMELAS RAFINESQUE. Bulletin of Environmental Contamination and Toxicology 39:869-876
Data Type:	Other; Aquatic; Fish
Hero ID:	3587456

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ 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.

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

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†† 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:	Thiébaud, H.,Merlin, G.,Capovilla, M. P.,Blake, G.. 1994. Fate of a volatile chlorinated solvent in indoor aquatic microcosms: sublethal and static exposure to [14C]dichloromethane. Groupe pour l'Etude du Devenir de X"nobiotiques dans l'Environnement (GEDEXE). Ecotoxicology and Environmental Safety 28:71-81
Data Type:	Other; Aquatic; other mesocosm BCF
Hero ID:	3588425

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified.
	Metric 2:	Test Substance Source	Low	× 1	3	Source not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of methylene chloride was not provided.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Negative controls were used.
	Metric 5:	Negative Control Response	N/A		N/A	This is a fate study conducted in a mesocosm, not a toxicity test.
	Metric 6:	Randomized Allocation	N/A		N/A	This is a fate study conducted in a mesocosm, not a toxicity test.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A		N/A	
	Metric 12:	Testing at or Below Solubility Limit	N/A		N/A	This is a fate study conducted in a mesocosm, not a toxicity test.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Not indicated,
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	

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Study Citation:	Thiébaud, H.,Merlin, G.,Capovilla, M. P.,Blake, G.. 1994. Fate of a volatile chlorinated solvent in indoor aquatic microcosms: sublethal and static exposure to [14C]dichloromethane. Groupe pour l'Etude du Devenir de X"nobiotiques dans l'Environnement (GEDEXE). Ecotoxicology and Environmental Safety 28:71-81
Data Type:	Other; Aquatic; other mesocosm BCF
Hero ID:	3588425

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	Unacceptable	× 2	8	NO adverse outcome- This study analyzed the bioaccumulation/concentration factors of DCM.
	Metric 18:	Consistency of Outcome Assessment	N/A		N/A	No adverse outcome- This study analyzed the bioaccumulation/concentration factors of DCM.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	N/A		N/A	This study analyzed the bioaccumulation/ concentration factors of DCM.
	Metric 20:	Outcomes Unrelated to Exposure	N/A		N/A	This study analyzed the bioaccumulation/ concentration factors of DCM.
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	N/A		N/A	This study analyzed the bioaccumulation/ concentration factors of DCM, not a toxicity test.
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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† 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.

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

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†† 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, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
Data Type:	Chronic (>21 days); Aquatic; Invertebrates
Hero ID:	3589368

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as methylene chloride.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source was a chemical supplier, Fisher Scientific.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was 99.9 percent.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	A seawater control was used.
	Metric 5:	Negative Control Response	High	× 1	1	The average control mortality was reported as 4.2 percent, with one developmental delay and no abnormalities.
	Metric 6:	Randomized Allocation	Low	× 1	3	No randomization described in the study report.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	The static test was conducted in glass tissue culture tubes, but its unclear whether these were capped, so loss of methylene chloride from volatilization was possible.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Test concentrations were nominal for this volatile substance and the test was static; its unclear whether the test vessels were capped to reduce volatilization.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	The embryo-larvae test was 12 days (embryos were exposed from 3 days to 15 days) in this static test.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Five concentrations plus the seawater control were tested with 24 embryos per test concentration.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were all below the water solubility of methylene chloride.
Domain 4: Test Organism						

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Study Citation: Rayburn, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
Data Type: Chronic (>21 days); Aquatic; Invertebrates
Hero ID: 3589368

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Grass shrimp were collected from an estuary of unknown water quality and not cultured for laboratory testing. However, average control morality was 4.2 percent and 1 developmental delay, with no abnormalities, so these appear acceptable.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	The grass shrimp were acclimated to the aquaria for at least 2 weeks.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	There were 3 replicates of 24 shrimp embryos per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Shrimp embryos were examined daily for heartbeat and morphological abnormalties, hatching, and developmental delays.
	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 tubes were incubated at 27C and rotated at 60 rpm, Details for water parameters were sparse, but control response indicates it was within normal ranges.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	Control responses indicate no outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	LC50s with confidence intervals calculated with Litchfield-Wilcox probit analysis.
	Metric 22:	Reporting of Data	High	× 2	2	
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			~~Unacceptable~~ ⟶		4.0	
Extracted			No			

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Study Citation:	Rayburn, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
Data Type:	Chronic (>21 days); Aquatic; Invertebrates
Hero ID:	3589368

Domain	Metric	Rating†	MWF⋆	Score	Comments††

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Study Citation:	Rayburn, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	3589368

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as methylene chloride.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source was a chemical supplier, Fisher Scientific.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was 99.9 percent.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	A seawater control was used.
	Metric 5:	Negative Control Response	High	× 1	1	The average control mortality was reported as 4.2 percent, with one developmental delay and no abnormalities.
	Metric 6:	Randomized Allocation	Low	× 1	3	No randomization described in the study report.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Unacceptable	× 2	8	The static test was conducted in glass tissue culture tubes, but it is unclear whether the tubes were capped and no further steps to reduce loss of methylene chloride from volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Test concentrations were nominal for this volatile substance and the test was static. The test vessels are tissue culture tubes, but unclear whether tubes were capped and airspace allows for volatilization of methylene chloride, so test concentrations were lower than reported.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	The embryo-larvae test was 96-hours
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Five concentrations plus the seawater control were tested with 24 embryos per test concentration.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were all below the water solubility of methylene chloride.
Domain 4: Test Organism						

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Study Citation:	Rayburn, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	3589368

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Organism Characteristics	Medium	× 2	4	Grass shrimp were collected from an estuary of unknown water quality and not cultured for laboratory testing. However, average control morality was 4.2 percent and 1 developmental delay, with no abnormalities, so these appear acceptable.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	The grass shrimp were acclimated to the aquaria for at least 2 weeks.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	There were 3 replicates of 24 shrimp embryos per test concentration.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Shrimp embryos were examined daily for heartbeat and morphological abnormalties.
	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 tubes were incubated at 27C and rotated at 60 rpm, Details for water parameters were sparse, but control response indicates it was within normal ranges.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	Control responses indicate no outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	LC50s with confidence intervals calculated with Litchfield-Wilcox probit analysis.
	Metric 22:	Reporting of Data	High	× 2	2	
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			~~Unacceptable~~ ⟶		4.0	
Extracted			No			

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Study Citation:	Rayburn, J. R.,Fisher, W. S.. 1999. Developmental toxicity of copper chloride, methylene chloride, and 6-aminonicotinamide to embryos of the grass shrimp Palaemonetes pugio. Environmental Toxicology and Chemistry 18:950-957
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Domain	Metric	Rating†	MWF⋆	Score	Comments††

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⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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: Birge, W. J.,Black, J. A.,Kuehne, R. A.. 1980. Effects of Organic Compounds on Amphibian Reproduction.
Data Type: Acute (0-96 hour); Aquatic; other Amphibians
Hero ID: 3616521

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source not indicated.
	Metric 3:	Test Substance Purity	Medium	× 1	2	All test substances were analytical or spectrophotometric grade, but specific purity for methylene chloride was not indicated.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Negative controls were 50 to 130 eggs.
	Metric 5:	Negative Control Response	High	× 1	1	The control embryo/larvae survival ranged from 82 to 98 percent.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomized allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Flow-through test in a closed chamber without head space to minimize volatilization, and daily analysis of test substance concentrations during testing.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	High	× 2	2	Gas chromatography was used to measure concentrations of methylene chloride in test vessels on a daily basis.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Eggs and larvae were exposed up to 4 days post-hatch using flow-through exposure concentrations. Appropriate for embryo-larval EC50 and LC50 determination.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Methylene chloride was tested at 5 concentrations per species with appropriate spacing of exposure concentrations.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Exposure concentrations tested were below methylene chloride's water solubility limit.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Amphibians obtained were appropriate for the embryo-larval test.

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Study Citation: Birge, W. J.,Black, J. A.,Kuehne, R. A.. 1980. Effects of Organic Compounds on Amphibian Reproduction.
Data Type: Acute (0-96 hour); Aquatic; other Amphibians
Hero ID: 3616521

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Fertilized eggs were either freshly obtained from the local fish hatchery, or by ovulation induction and fertilization of fresh eggs from frogs in the laboratory. All testing was initiated within 30 minutes of fertilization.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Study indicated single replicates of 50 to 130 eggs were used per methylene chloride exposure concentration for each of three amphibian species.
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	There were up to 130 eggs per 500 mL test chamber. Control survival was within normal limits, so this appears acceptable.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Control-adjusted embryo-larval survival with daily examination and removal of dead embryos/larvae.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	LC50, LC10 and LC1 values were adjusted for control mortality and teratogenicity.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	Control survival >80 percent and environmental conditions were within acceptable ranges.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	× 1	2	Control mortality ranged from 2 - 18 percent, however detailed control data was not provided.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Medium	× 1	2	Finney's probit analysis was used to obtain LC50, LC10 and LC1 values with 95 percent confidence limits; percent survival and mortality endpoints were corrected for control mortality, but control data was not reported.
	Metric 22:	Reporting of Data	Medium	× 2	4	Percent hatchability and survival of larvae reported in table format for each test concentration, in addition to LC50, LC10 and LC1. Detailed control mortality data was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.3	

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Study Citation:	Birge, W. J.,Black, J. A.,Kuehne, R. A.. 1980. Effects of Organic Compounds on Amphibian Reproduction.
Data Type:	Acute (0-96 hour); Aquatic; other Amphibians
Hero ID:	3616521

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	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 Volvulina steinii NIES545. 18:43-46
Data Type:	Other; Aquatic; other Algae; Chlorophyll a
Hero ID:	3617103

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as dichloromethane.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source not specified
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not specified
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Controls were included.
	Metric 5:	Negative Control Response	High	× 1	1	Control responses were reported in figures.
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomized allocation not indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	The vessels were covered, according to methods of Arensberg et al., (1995) that authors referred to, however headspace remained allowing for volatilization of methylene chloride.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Test concentrations were reported in terms of nominal concentrations and, according to methods of Arensberg et al., (1995) loss of volatile solvent possible into test vessel headspace.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Treatment cultures were exposed for 10 days under static conditions.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Including the controls, there were 7 exposure concentrations (nominal) that were spaced to detect a concentration-response relationship.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Testing was well below the water solubility limit of methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Chlorella vulgaris and Selenestrum capricornutum (now Raphidocelis subcapitata) are well studied static algae species; Volvulina steiniis is a flagellar algae.

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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, Chlorella vulgaris NIES227, Selenastrum capricornutum NIES35, and Volvulina steinii NIES545. 18:43-46
Data Type:	Other; Aquatic; other Algae; Chlorophyll a
Hero ID:	3617103

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	Algae were incubated prior to addition of test material, but details were not provided.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	× 1	2	Five replicates at two different volumes (1 mL and 10 mL) for each algae species were tested, with initial concentrations not specified.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	Conditions were adequate as evidenced by control vessel algal growth.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	Low	× 2	6	Chlorophyll a light absorbance was reported for all treatments and controls to measure growth, but is not considered an accurate method for measuring algal biomass.
	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 inconsistencies in the test design and methodology were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	
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	Chlorophyll a absorbance as a measure of growth was reported for all test concentrations, but an EC50 value was not calculated relative to the absorbance; presented in figures only.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	V. steinii test was repeated to confirm results.
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

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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, Chlorella vulgaris NIES227, Selenastrum capricornutum NIES35, and Volvulina steinii NIES545. 18:43-46
Data Type:	Other; Aquatic; other Algae; Chlorophyll a
Hero ID:	3617103

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Wilson, J. E. H.. 1998. Developmental Arrest in Grass Shrimp Embryos Exposed to Selected Toxicants.
Data Type:	Aquatic; Invertebrates
Hero ID:	3617783

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not indicated.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity is not defined which meant the review was unable to calculate the test concentrations in terms of parts per million.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Seawater controls were used.
	Metric 5:	Negative Control Response	High	× 1	1	Results for negative controls reported.
	Metric 6:	Randomized Allocation	High	× 1	1	Stratified randomization method used.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	Flasks were covered parafilm to reduce evaporation, and authors specified a static exposure regime, but headspace in flasks allows volatilization of methylene chloride.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Measurement of Test Substance Concentration	Low	× 2	6	Test was acute, but concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	Exposure for 96-hours under static conditions.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Five exposure concentrations spaced exponentially.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Testing well below water solubility for methylene chloride.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Lab-cultured grass shrimp embryos well characterized.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Lab-cultured embryos for controls and treatment groups appeared given same pre-treatment conditions prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	30 embryos per flask, with two replications.

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Study Citation:	Wilson, J. E. H.. 1998. Developmental Arrest in Grass Shrimp Embryos Exposed to Selected Toxicants.
Data Type:	Aquatic; Invertebrates
Hero ID:	3617783

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Embryos assessed for NOEC and LOEC for mortality, developmental delays/arrest and malformation at 8 developmental stages through larval hatching.
	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	None reported, and mortality/development abnormalities were less than 10 percent in controls.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	Mortality and developmental abnormalities were less than 10 percent for controls.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Low	× 1	3	No statistical analysis were referenced, results were reported in terms of NOAEC/LOAEC.
	Metric 22:	Reporting of Data	Medium	× 2	4	Results for developmental delays, embryonic time to develop, and mortality were reported for all test chemicals.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as methylene chloride.
	Metric 2:	Test Substance Source	Low	× 1	3	A source was not provided.
	Metric 3:	Test Substance Purity	High	× 1	1	The authors described the chemical purity as "reagent grade"
Domain 2: Test Design						
	Metric 4:	Negative Controls	Medium	× 2	4	Authors referred to a control, but additional details were not reported.
	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	Test systems were sealed with no headspace to eliminate volatilization.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	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 and vessels were sealed with no headspace.
	Metric 10:	Exposure Duration and Frequency	Medium	× 1	2	The test duration was 48 hours, so results are not comparable with 72 to 96 hours for algal testing.
	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	Test concentrations were not provided, but the LC50 is below the water solubility of DCM.
Domain 4: Test Organism						

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Data Type:	Acute (0-96 hour); Aquatic; Plants
Hero ID:	3617867

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Organism Characteristics	High	× 2	2	Pseudokirchneriella subcapitata is a well-known algal species.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	15,000 algal cells/mL inoculum at test initiation, with 3 replicates.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Outcome assessed at percent inhibition using an electronic particle counter to evaluate cell density at test initiation for controls and treatments.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	percent Inhibition was calculated relative to controls.
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	High	× 2	2	Authors conducted concentration checks where a nominal test concentration was compared to a measured concentration from a vessel without algae. The test was repeated if these concentrations differed greater than 6 percent.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	percent inhibition was reported relative to control concentrations.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	
	Metric 22:	Reporting of Data	Medium	× 2	4	Results did not include raw data.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Data Type:	Acute (0-96 hour); Aquatic; Plants
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Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Kramer, V. C.,Schnell, D. J.,Nickerson, K. W.. 1983. Relative Toxicity of Organic Solvents to Aedes aegypti Larvae. 42:285-287
Data Type:	Acute (0-96 hour); Aquatic; other Insect-larvae 4-hour LC50
Hero ID:	3661235

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test chemical was identified as methylene chloride.
	Metric 2:	Test Substance Source	Medium	× 1	2	Chemicals were described as "reagent grade" but no further information was provided.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Chemicals were described as "reagent grade" but no further information was provided.
Domain 2: Test Design						
	Metric 4:	Negative Controls	High	× 2	2	Controls were used in this test.
	Metric 5:	Negative Control Response	High	× 1	1	There was no mortality observed in the controls.
	Metric 6:	Randomized Allocation	N/A		N/A	A single replicate of 10-20 larvae were transferred from stock maintained in glass beakers/jars to 50 mL beakers for testing.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	No specific deficiencies were identified, but scarce details were provided regarding the test preparation.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure was described as a bioassay, which was repeated three times for each test chemical, but the study report lacked sufficient details describing the bioassay procedure.
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	Test concentrations were not provided, only endpoints were provided.
	Metric 10:	Exposure Duration and Frequency	Low	× 1	3	4 Hour duration were sufficient for the needs of the authors, as they were looking to measure acute toxicity. But this limited duration has low utility outside of the results of this study.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	Details about the exposure concentrations were unclear.
	Metric 12:	Testing at or Below Solubility Limit	N/A		N/A	Details about the exposure concentrations were unclear.
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Larva of Aedis aegypti (mosquitos).

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Study Citation:	Kramer, V. C.,Schnell, D. J.,Nickerson, K. W.. 1983. Relative Toxicity of Organic Solvents to Aedes aegypti Larvae. 42:285-287
Data Type:	Acute (0-96 hour); Aquatic; other Insect-larvae 4-hour LC50
Hero ID:	3661235

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14:	Acclimitization and Pretreatment Conditions	Medium	× 1	2	No Acclimatization period reported, but embryos obtained from research laboratory and hatched. There were no mortalities in controls indicating adequate pretreatment conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	× 1	3	A single replicate of 10-20 individuals per chemical, number not specified for DCM.
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	Individuals larvae were raised on dog food in baby food jars prior to testing. Although there is a lack of guideline for mosquito larvae maintenance, the controls had no mortaility, indicating that conditions were adequate for the test period.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	
	Metric 18:	Consistency of Outcome Assessment	Low	× 1	3	Details about the test procedure were not provided
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	× 2	6	The authors did not provide enough information to allow for a comparison of the environmental conditions or other non treatment related factors.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Unacceptable	× 1	4	Data were not provided, description of the statistical analyses were not provided.
	Metric 22:	Reporting of Data	Low	× 2	6	Only endpoint values (LC50s) were provided.
	Metric 23:	Explanation of Unexpected Outcomes	High	× 1	1	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Data Type:	Acute (0-96 hour); Aquatic; other Insect-larvae 4-hour LC50
Hero ID:	3661235

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.

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Study Citation:	Kramer, V. C.,Schnell, D. J.,Nickerson, K. W.. 1983. Relative Toxicity of Organic Solvents to Aedes aegypti Larvae. 42:285-287
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	3661235

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Chemical is specified as Methylene Chloride, but properties of the test material were not specified
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Chemicals were described as "reagent grade" but no further information was provided.
	Metric 3:	Test Substance Purity	Unacceptable	× 1	4	Chemicals were described as "reagent grade" but no further information was provided.
Domain 2: Test Design						
	Metric 4:	Negative Controls	Medium	× 2	4	Negative control was used, no mortality was reported. No data provided
	Metric 5:	Negative Control Response	High	× 1	1	Negative control was used, no mortality was reported. No data provided
	Metric 6:	Randomized Allocation	N/A		N/A	
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	Low	× 2	6	No specific deficiencies were identified, but scarce details were provided regarding the test preparation.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Exposure was described as a bioassay, which was repeated three times for each test chemical, but the study report lacked sufficient details describing the bioassay procedure.
	Metric 9:	Measurement of Test Substance Concentration	Unacceptable	× 2	8	test concentrations were not provided, only endpoints were provided.
	Metric 10:	Exposure Duration and Frequency	Low	× 1	3	4 Hour duration were sufficient for the needs of the authors, as they were looking to measure acute toxicity. But this limited duration has low utility outside of the results of this study.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Unacceptable	× 1	4	Details about the exposure concentrations were unclear.
	Metric 12:	Testing at or Below Solubility Limit	N/A		N/A	
Domain 4: Test Organism						

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Study Citation: Kramer, V. C.,Schnell, D. J.,Nickerson, K. W.. 1983. Relative Toxicity of Organic Solvents to Aedes aegypti Larvae. 42:285-287
Data Type: Acute (0-96 hour); Aquatic; Invertebrates
Hero ID: 3661235

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 13:	Test Organism Characteristics	High	× 2	2	A. aegypti larvae employed were derived from a colony obtained from the Gulf Coast Mosquito Research Laboratory, U.S. Department of Agriculture, Lake Charles, Louisiana.
	Metric 14:	Acclimitization and Pretreatment Conditions	Low	× 1	3	No Acclimatization period reported.
	Metric 15:	Number of Organisms and Replicates per Group	Unacceptable	× 1	4	Number of individuals/replicate not specificed
	Metric 16:	Adequacy of Test Conditions	Low	× 1	3	Individuals larvae were raised on dog food in baby food jars. Lack of guideline for mosquito larvae means that it was uncertain whether this was sufficient or not.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	The purpose of this study was to assess the suitability of solvents for use in bioassay experiments. The authors were able to get a comparative response for a variety of substances.
	Metric 18:	Consistency of Outcome Assessment	Low	× 1	3	Details about the test procedure were not provided
Domain 6: Confounding / Variable Control						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	× 2	6	The authors did not provide enough information to allow for a comparison of the environmental conditions or other non treatment related factors.
	Metric 20:	Outcomes Unrelated to Exposure	N/A		N/A	
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Unacceptable	× 1	4	Data were not provided, description of the statistical analyses were not provided.
	Metric 22:	Reporting of Data	Unacceptable	× 2	8	No raw data were provided
	Metric 23:	Explanation of Unexpected Outcomes	N/A		N/A	
Overall Quality Determination‡			Unacceptable		4.0	
Extracted			No			

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Study Citation:	Kramer, V. C.,Schnell, D. J.,Nickerson, K. W.. 1983. Relative Toxicity of Organic Solvents to Aedes aegypti Larvae. 42:285-287
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	3661235

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** 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, seven 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	Minnesota Mining & Mfg Co. 1979. 96-HOUR LC50 AQUATIC TEST ON FATHEAD MINNOWS WITH COVER LETTER.
Data Type:	Acute (0-96 hour); Aquatic; other Mixture 63 percent DCM; fathead minnow
Hero ID:	4213679

Domain		Metric	Rating†	MWF⋆	Score	Comments
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Unacceptable	× 2	8	Mixture with 63 percent methylene chloride
	Metric 2:	Test Substance Source	N/A		N/A	
	Metric 3:	Test Substance Purity	N/A		N/A	
Domain 2: Test Design						
	Metric 4:	Negative Controls	N/A		N/A	
	Metric 5:	Negative Control Response	N/A		N/A	
	Metric 6:	Randomized Allocation	N/A		N/A	
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	N/A		N/A	
	Metric 8:	Consistency of Exposure Administration	N/A		N/A	
	Metric 9:	Measurement of Test Substance Concentration	N/A		N/A	
	Metric 10:	Exposure Duration and Frequency	N/A		N/A	
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	N/A		N/A	
	Metric 12:	Testing at or Below Solubility Limit	N/A		N/A	
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	N/A		N/A	
	Metric 14:	Acclimitization and Pretreatment Conditions	N/A		N/A	
	Metric 15:	Number of Organisms and Replicates per Group	N/A		N/A	
	Metric 16:	Adequacy of Test Conditions	N/A		N/A	
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	N/A		N/A	
	Metric 18:	Consistency of Outcome Assessment	N/A		N/A	

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Data Type:	Acute (0-96 hour); Aquatic; other Mixture 63 percent DCM; fathead minnow
Hero ID:	4213679

Domain	Metric	Rating†	MWF⋆	Score	Comments
Domain 6: Confounding / Variable Control					
	Metric 19: Confounding Variables in Test Design and Procedures	N/A		N/A	
	Metric 20: Outcomes Unrelated to Exposure	N/A		N/A	
Domain 7: Data Presentation and Analysis					
	Metric 21: Statistical Methods	N/A		N/A	
	Metric 22: Reporting of Data	N/A		N/A	
	Metric 23: Explanation of Unexpected Outcomes	N/A		N/A	
Overall Quality Determination‡		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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.

Study Citation:	E I Dupont Denemours & Co Inc. 1987. FLOW-THROUGH ACUTE 96-HOUR LC50 OF METHYLENE CHLORIDE TO RAINBOW TROUT (SANITIZED).
Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	4213816

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CAS.
	Metric 2:	Test Substance Source	Low	× 1	3	Source not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative Controls	Medium	× 2	4	Negative control was used. A small amount of test substance was detected in the control but it does not seemed to have had an effect on the fish.
	Metric 5:	Negative Control Response	High	× 1	1	Control response was reported and had 0 percent mortality.
	Metric 6:	Randomized Allocation	High	× 1	1	Ten juvenile rainbow trout were randomly placed in the test vessels.
Domain 3: Exposure Characterization						
	Metric 7:	Experimental System/Test Media Preparation	High	× 2	2	Experimental system was described and appropriate. Although it was not mentioned whether the system was enclosed, the test was flow through and test substance was renewed every 20 minutes. This is important for a volatile chemical like methylene chloride.
	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	Measurements of the test substance in each test concentration and in the control were taken every 24 hours.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	The duration, 96 hours, and exposure frequency (flow-through with renewal every 20 min) are recommended durations and frequency according to OECD TG 203.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	Authors reported 6 exposure groups, which meet the minimum recommended in OECD TG 203, and spacing was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	× 1	1	Test concentrations were well below the solubility of methylene chloride.

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	4213816

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Rainbow trout are a recommended species in OECD TG 203. Fish were from Haskell lab stock.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Fish were held for about 94 days before the test, which meets the min number of days to hold the fish recommended in OECD TG 203. N treatment of the fish for diseases was required during the holding period.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Ten fish per test concentration were used. OECD TG 203 recommends at least 7.
	Metric 16:	Adequacy of Test Conditions	Medium	× 1	2	The 3.8 g fish/L loading may be acceptable given that this is a flow-through tests (OECD TG 203 recommends 1 g fish/L for static tests but could be higher for flow-through). Only minor uncertainties about housing conditions, not likely to have a substantial impact on the results, given the 0 percent mortality observed in the controls.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	This test was able to derive an LC50.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were 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 outcomes unrelated to exposure were not reported for each study group. Only substantial differences between groups was noted.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	Low	× 1	3	The statistical method was not described clearly.
	Metric 22:	Reporting of Data	High	× 2	2	The results of the test were reported for each study group in a table, and any effects besides mortality were described in the text.

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Data Type:	Acute (0-96 hour); Aquatic; Fish
Hero ID:	4213816

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Explanation of Unexpected Outcomes	High	× 1	1	Authors noted the small concentrations of test chemical in the control, and hypothesized about the source, but this does not seem to have affected the results.
Overall Quality Determination‡		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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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:	E I Dupont Denemours & Co Inc. 1987. DAPHNIA MAGNA STATIC ACUTE 48-HOUR EC50 OF METHYLENE CHLORIDE (SANITIZED).
Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	4213817

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical identified by name and CAS.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported.
	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	Study included a control using water.
	Metric 5:	Negative Control Response	High	× 1	1	Control response was reported and no immobilization was observed in the control.
	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	Authors did not report covering vessels and methylene chloride is a very volatile chemical. However, they did measure concentrations at day 0 and day 2. Contaminants were also noted, leaving uncertainties about what was causing the observed toxicity.
	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 day 0 and day 2, and an average of those measurements was used at each concentration level.
	Metric 10:	Exposure Duration and Frequency	High	× 1	1	The test was a static 48-hour tests which is recommended by OECD TG 202 for acute daphnia tests.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	High	× 1	1	At least 5 test concentrations were used, and authors were able to derive an EC50 with the concentrations tested.
	Metric 12:	Testing at or Below Solubility Limit	Medium	× 1	2	The test concentrations are far below the solubility limit for methylene chloride. However, the divergence between nominal and measured concentrations increases with concentration level, creating minor uncertainties.

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	4213817

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Organism Characteristics	High	× 2	2	Daphnia magna are a recommended species in OECD TG 202, and the source was reported as Haskell Laboratory-bred stock.
	Metric 14:	Acclimitization and Pretreatment Conditions	High	× 1	1	Authors reported a 24 hour acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	High	× 1	1	Authors mentioned using 4 replicates of 5 daphnia per concentration which is recommended by OECD TG 202.
	Metric 16:	Adequacy of Test Conditions	High	× 1	1	Test conditions were appropriate for daphnia.
Domain 5: Outcome Assessment						
	Metric 17:	Outcome Assessment Methodology	High	× 2	2	Authors derived an EC50.
	Metric 18:	Consistency of Outcome Assessment	High	× 1	1	Details of outcome assessment were reported and consistent 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 group in the environmental conditions or other factors that could influence the outcome of the assessment.
	Metric 20:	Outcomes Unrelated to Exposure	High	× 1	1	No outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 21:	Statistical Methods	High	× 1	1	Authors reported conducting a probit analysis.
	Metric 22:	Reporting of Data	High	× 2	2	Authors reported response rates for every test concentration and replicate.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	× 1	2	Authors included a table with the number of daphnia exhibiting immobilization, and said that it was cumulative. However, after 48 hours, there were fewer immobilized daphnia than at 24 hours for the 180 mg/L concentration. Authors didn't explain this inconsistency.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Data Type:	Acute (0-96 hour); Aquatic; Invertebrates
Hero ID:	4213817

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ 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.

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

where High: ≥ 1 to < 1.7; Medium: ≥ 1.7 to < 2.3; Low: ≥ 2.3 to ≤ 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.