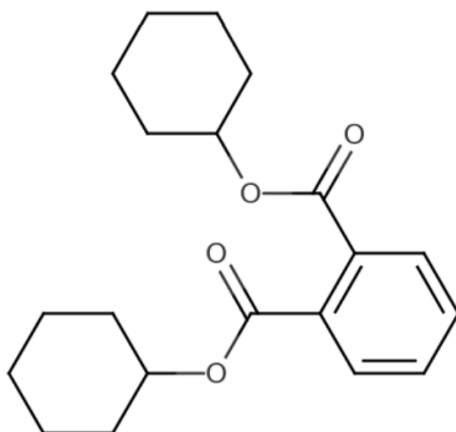


**Data Quality Evaluation Information for  
Environmental Hazard for  
Dicyclohexyl Phthalate (DCHP)  
(1,2- Benzenedicarboxylic acid, 1,2-dicyclohexyl ester)**

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

**CASRN: 84-61-7**



*December 2025*

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

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

# Table of Contents

HERO ID	Reference	Page
<b>Dicyclohexyl Phthalate</b>		
<b>Habitat: Aquatic (freshwater)</b>		
<b>Taxa: Vertebrates</b>		
<i>Oryzias latipes</i>		
11803931	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka ( <i>Oryzias latipes</i> ) (translation).	4
<i>Silurana tropicalis</i>		
3230411	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.	7
<b>Taxa: Invertebrates</b>		
<i>Daphnia magna</i>		
11803964	NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on <i>Daphnia magna</i> (translation).	13
11803962	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).	17
<i>Macrobrachium rosenbergii</i>		
789598	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . <i>Aquatic Toxicology</i> 64(1):25-37.	23
<b>Taxa: Plants (Non-vascular)</b>		
<i>Selenastrum capricornutum</i>		
11803966	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae ( <i>Selenastrum capricornutum</i> ) (translation).	29

<b>Study Citation:</b>	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka ( <i>Oryzias latipes</i> ) (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803931			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The CASRN was included in the Japanese pdf.	
	Metric 2: Test Substance Source	Low	The source of the DCHP was not reported in the ECHA document, and was unable to be determined in the Japanese pdf.	
	Metric 3: Test Substance Purity	High	The purity appears to be 99.9% (shown in the Japanese pdf).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	A pure control and a solvent control were both used in this study.	
	Metric 5: Negative Control Response	High	Mortality in the control did not exceed 10% at the duration of the test.	
	Metric 6: Randomized Allocation	Low	It was not reported how fish were allocated to the test groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	The test system was semi-static with water renewals every 24 hours. Each test solution was covered by a Teflon sheet. Details on test media preparation were not reported.	
	Metric 8: Consistency of Exposure Administration	High	The volume of test solution per test concentration was five liters. The test temperature was kept at 24C with a 16L:8D photoperiod. Test concentrations were measured and were consistent throughout the study.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The DCHP was measured using HPLC. Measured concentrations can be found in Table 1 in the Japanese pdf.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was 96 hours, which is suitable for a fish acute toxicity test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Low	There was only one DCHP test concentration reported with the results. The ECHA document mentions that the 2mg/L test concentration was the highest dispersible concentration used. However, there is some uncertainty surrounding test concentrations used for this study. In the Japanese pdf there are only the two controls and the one test concentration (2 mg/L) shown in the results tables. In the executive summary of the ECHA document it mentions fish were exposed to a range of concentrations from 10 to 100 mg/L. But further up in the document it mentions that only a test concentration of 2 mg/L was able to get into solution and measured throughout the test. LC50s were calculated and those results shown, but they were only based on the one measured test concentration. There were no reported mortalities in the test so all the LC50s were greater than the 2 mg/L measured test concentration.	
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<b>Study Citation:</b>	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka ( <i>Oryzias latipes</i> ) (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803931			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	Low	There is mention of using test concentrations ranging from 10 to 100 mg/L, but no further information on that regarding why they weren't shown in the results. According to further information provided in the ECHA document, it appears there was a solubility issue with those higher test concentrations if they were truly tried in the exposure. The 2 mg/L test concentration was reported to be the highest dispersible concentration and the only one shown in the results. The solvent concentration was reported as 100 mg/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test fish general weight and length were reported. The fish were obtained from Sankyo Suisan Co.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	The ECHA document mentioned that the test fish were acclimated for almost two months prior to test start. Test organisms were visually healthy and normal before the test start. The ECHA document has text "acclimatization conditions within 12 days before exposure are shown as follows", but the information shown below that appears to be for the test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Ten fish were used per test concentration, but there was only one replicate container per concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be healthy, and the mortality rate was below 5% during the acclimation period. Ten fish per test concentration container seemed suitable for the acute test. During the test dead fish were removed to prevent deteriorating water quality. The fish were not fed during the test. No aeration was used but water was renewed every 24 hours. Water quality conditions were measured and were adequate during the exposure. Measured temperature, dissolved oxygen and pH values were shown in tables 6, 7, and 8 (respectively) in the Japanese pdf. The ECHA document did report the water media type to be 'saltwater' but that is unlikely as medaka are a freshwater species, and it was mentioned further down in that document (Executive summary) that fish were exposed to test concentrations in freshwater.
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed every 24 hours for the duration of the 96h test. This was adequately described in the ECHA document.
	Metric 18:	Consistency of Outcome Assessment	High	The ECHA document reported that mortality was assessed across all exposure concentrations every 24 hours.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Environmental conditions were adequate and consistent throughout the exposure. Fish were acclimated prior to the start of the test. Though, it is unclear what the actual water conditions were during the acclimation period.
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<b>Study Citation:</b>	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka ( <i>Oryzias latipes</i> ) (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803931			
Domain	Metric	Rating	Comments	
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	Even though in Table 3 of the Japanese pdf there are columns shown for 95% confidence limits and statistical method, no information is reported. Only one test concentration was used to calculate LC50s and there were no mortalities in any of the exposure concentrations, so statistical analysis was not necessary.
	Metric 22:	Reporting of Data	High	In the Japanese pdf, mortality data was shown in Tables 2 and 4. The calculated LC50 data was shown in Table 3. Mortality results were also briefly described in the ECHA document.
	Metric 23:	Explanation of Unexpected Outcomes	High	There was no reporting of any unexpected outcomes. Measures of variability were not necessary because there was only one test concentration shown and no mortalities observed in either the controls or the test concentration.
Additional Comments:	This evaluation is for the mortality assessment after medaka were exposed to DCHP for 96 hours. Please note, this is a Japanese study and there was not a professionally translated English version available for this evaluation, so instead it is based off of the Japanese pdf and an accompanying ECHA document. There are some discrepancies in the text regarding the actual amount of test concentrations used and the adequacy of basing LC50 values off of the one usable test concentration. The ECHA document mentioned that 'observed signs of toxicity' were also recorded every 24 hours for the duration of the test. Results were shown for this in Table 5 in the Japanese pdf (no toxicological symptoms were observed for any treatment at any timepoint). From both these documents it is unclear what specific symptoms were evaluated, so therefore no separate outcome inventory form was completed for this. A professionally translated version of the study may provide more details for this.			

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

<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	3230411			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHP was identified by CAS no.	
	Metric 2: Test Substance Source	Low	The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported to be 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a water control and a solvent control (0.82% DMSO).	
	Metric 5: Negative Control Response	High	The negative control response was reported in Table 1 and was adequate for the outcome of interest.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test media. The type of experimental container material used (glass vs plastic) was not reported.	
	Metric 8: Consistency of Exposure Administration	Low	It was reported that new FETAX solution was used daily and the solutions were re-spiked at this time, but test chambers were not described.	
	Metric 9: Measurement of Test Substance Concentration	High	Study authors reported using GC-MS to measure the test substances.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure was 72h, and study authors reported this was shorter than the typical 96h test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups and the spacing was adequate to observe a response.	
	Metric 12: Testing at or Below Solubility Limit	High	DMSO was used a vehicle solvent at 0.82%. A solvent control was used and the response was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Adult frogs were from Queen’s University Animal Care Facility in Kingston, ON, Canada. Embryos were obtained from the adults and used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms used in each test concentration is reported in Table 1.	
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<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.		
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae		
<b>Health Outcome:</b>	Mortality		
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)		
<b>HERO ID:</b>	3230411		
Domain	Metric	Rating	Comments
Domain 5: Outcome Assessment			
Metric 16:	Adequacy of Test Conditions	Low	Embryos were kept at 26 C in FETAX solution. Little other information was provided on the environmental conditions such as photoperiod, adult feeding regimen, and loading rate.
Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality.
Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Embryos were monitored daily for mortalities, and dead embryos removed at this point.
Domain 6: Confounding / Variable Control			
Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis			
Metric 21:	Statistical Methods	High	Statistical methods were described in detail in the "Data Analysis" section of the paper.
Metric 22:	Reporting of Data	High	Data for the exposure related findings and the control response are reported in Table 1 and are adequate for the outcome of interest.
Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Table 1.
Additional Comments:	This portion of the evaluation was on the effect of DCHP on embryo mortality in <i>S. tropicalis</i> . Mortality was selected as the outcome of interest. There is supplemental material that was not included with the Distiller download. This contains more data regarding the mechanistic outcomes in the paper.		
<b>Overall Quality Determination</b>		<b>High</b>	



<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	3230411			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHP was identified by CAS no.	
	Metric 2: Test Substance Source	Low	The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported to be 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a water control and a solvent control (0.82% DMSO).	
	Metric 5: Negative Control Response	Medium	The negative control response was reported in Tables 1 and 4. The solvent control had significantly more malformations and underdeveloped individuals when compared to the water controls.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test media. The type of experimental container material used (glass vs plastic) was not reported.	
	Metric 8: Consistency of Exposure Administration	Low	It was reported that new FETAX solution was used daily and the solutions were re-spiked at this time, but test chambers were not described.	
	Metric 9: Measurement of Test Substance Concentration	High	Study authors reported using GC-MS to measure the test substances.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure was 72h, and study authors reported this was shorter than the typical 96h test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups and the spacing was adequate to observe a response.	
	Metric 12: Testing at or Below Solubility Limit	High	DMSO was used a vehicle solvent at 0.82%. A solvent control was used and the response was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Adult frogs were from Queen’s University Animal Care Facility in Kingston, ON, Canada. Embryos were obtained from the adults and used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms used in each test concentration is reported in Tables 1 and 4.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	3230411			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	Low	Embryos were kept at 26 C in FETAX solution. Little other information was provided on the environmental conditions such as photoperiod, adult feeding regimen, and loading rate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–growth and malformations.	
	Metric 18: Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Embryos were assessed according the "Atlas of Abnormalities;" and were performed blinded.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	High	Statistical methods were described in detail in the "Data Analysis" section of the paper.	
	Metric 22: Reporting of Data	High	Data for the exposure related findings and the control response are reported in Tables 1 and 4 and are adequate for the outcome of interest.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Tables 1 and 4.	
Additional Comments:	This portion of the evaluation was on the effect of DCHP on embryo development and malformations in <i>S. tropicalis</i> . Development/growth was selected as the outcome of interest. There is supplemental material that was not included with the Distiller download. This contains more data regarding the mechanistic outcomes in the paper.			
Overall Quality Determination		High		

<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	3230411			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHP was identified by CAS no.	
	Metric 2: Test Substance Source	Low	The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.	
	Metric 3: Test Substance Purity	High	The purity was reported to be 99%.	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Study authors reported the use of a water control and a solventcontrol (0.82% DMSO).	
	Metric 5: Negative Control Response	Medium	The negative control response was reported in Figure 1. There were several cases in which the solvent control and the water control responses were significantly different.	
	Metric 6: Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Low	Limited details were provided on the preparation of the test media.	
	Metric 8: Consistency of Exposure Administration	Low	It was reported that new FETAX solution was used daily and the solutions were re-spiked at this time, but test chambers were not described.	
	Metric 9: Measurement of Test Substance Concentration	High	Study authors reported using GC-MS to measure the test substances.	
	Metric 10: Exposure Duration and Frequency	Medium	The exposure was 72h, and study authors reported this was shorter than the typical 96h test.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	High	There were five exposure groups and the spacing was adequate to observe a response.	
	Metric 12: Testing at or Below Solubility Limit	High	DMSO was used as the vehicle solvent at 0.82%. A solvent control was used, and the response was appropriate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	Adult frogs were from Queen’s University Animal Care Facility in Kingston, ON, Canada. Embryos were obtained from the adults and used for this study.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	It was not reported if the organisms were acclimated.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	The number of organisms used in each test concentration is reported in Tables 1 and 4.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	Mathieu-Denoncourt, J., Martyniuk, C. J., Loughery, J. R., Yargeau, V., Solla, de, S. R., Langlois, V. S. (2016). Lethal and sublethal effects of phthalate diesters in <i>Silurana tropicalis</i> larvae. <i>Environmental Toxicology and Chemistry</i> 35(10):2511–2522.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae			
<b>Health Outcome:</b>	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	3230411			
Domain	Metric	Rating	Comments	
	Metric 16:	Adequacy of Test Conditions	Low	Embryos were kept at 26C in FETAX solution. Little other information was provided on the environmental conditions such as photoperiod, adult feeding regimen, and loading rate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mRNA expression/mechanistic outcomes.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described in detail in the "Data Analysis" section of the paper.
	Metric 22:	Reporting of Data	High	Data for the exposure related findings and the control response are reported in Figures 1 and 2, and were adequate for the outcomes of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in Figure 1.
<b>Additional Comments:</b>	This portion of the evaluation was on the effect of DCHP on gene expression and the mechanisms underlying the toxicity. The mechanistic outcomes for biomarkers and cell signaling were chosen. There is supplemental material that was not included with the Distiller download. This contains more data regarding the mechanistic outcomes in the paper.			
<b>Overall Quality Determination</b>		<b>High</b>		

<b>Study Citation:</b>	NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803964			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHP was identified by CASRN.	
	Metric 2: Test Substance Source	Low	The source of the DCHP was not reported in the ECHA, and was unable to be determined in the Japanese PDF.	
	Metric 3: Test Substance Purity	High	The purity appears to be 99.9% (seen in the Japanese pdf).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both a solvent control and a control without any chemicals were used in this study.	
	Metric 5: Negative Control Response	High	The control and solvent control results of the preliminary test are reported in the ECHA document and are adequate for the outcome of interest.	
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	It was reported the test was conducted in a static system. The solution in the test containers was covered with a Teflon sheet. The test media preparation was not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	The test was conducted in 100 mL of test solution in a static system kept at 20C with a 16L:8D photoperiod. There is some uncertainty if the exposure details given are also relevant to the preliminary test	
	Metric 9: Measurement of Test Substance Concentration	Low	It was not reported if the DCHP was measured at any point in the preliminary test.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be 48h, which is typical of an acute toxicity test conducted with <i>Daphnia</i> .	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only three test concentrations, which is lower than is typical. However, this was for a preliminary test, so it is acceptable.	
	Metric 12: Testing at or Below Solubility Limit	Medium	One test concentration slightly exceeded the water solubility limit, but a solvent was used to aid in dissolution. The concentration of the solvent was not reported.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The <i>Daphnia magna</i> were reported to be from the National Institute for Environmental Studies. The organisms used in the test were >24h in age.	
	Metric 14: Acclimatization and Pretreatment Conditions	Low	Adult <i>Daphnia</i> were reported to be acclimated to test conditions for nearly a month (Nov 17-Dec 13, 1999) prior to collecting <24h old young for the start of the test. However, it was not reported what the specific water quality conditions were during the preliminary test, so a true comparison cannot be made.	
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<b>Study Citation:</b>	NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803964			
Domain	Metric	Rating	Comments	
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were ten organisms per test concentration with two replicates per treatment level for the preliminary test.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	The organisms were kept at 20C with a 16L:8D photoperiod below an illumination of 800 lux. They were not fed for testing, but they were fed <i>Chlorella vulgaris</i> during acclimation at 0.2mg of organic content per organism per day. The ECHA states the water used was saltwater, but this is unlikely as these are freshwater organisms. The water characteristics were not described in great deal anywhere.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–immobilization.
	Metric 18:	Consistency of Outcome Assessment	Low	The methods for determining immobilization were not reported in the ECHA document and were not in English in the Japanese document. Thus, the methods for determining immobilization are unclear at this point. It appears they were assessed at 24 and 48h, but little else is known.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	No immobilization was reported in the test concentration or in either of the controls, thus statistical analysis was not necessary.
	Metric 22:	Reporting of Data	High	Data for exposure related finding and control responses can be found in the ECHA document for the preliminary test.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.
Additional Comments:	This evaluation was for an acute toxicity test with <i>Daphnia magna</i> exposed to DCHP. Please note, this was a Japanese study in which a professional English translation was not available. This data was evaluated based off the tables in the Japanese PDF that were in English as well as an ECHA document for this particular study. Some metrics may have different ratings should a professionally translated English document become available.This evaluation was for the preliminary test.			

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

<b>Study Citation:</b>	NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803964			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHP was identified by CASRN.	
	Metric 2: Test Substance Source	Low	The source of the DCHP was not reported in the ECHA, and was unable to be determined in the Japanese PDF.	
	Metric 3: Test Substance Purity	High	The purity appears to be 99.9% (seen in the Japanese pdf).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	Both a solvent control and a control without any chemicals were used in this study.	
	Metric 5: Negative Control Response	High	The control and solvent control results are reported in Table 2 and are adequate for the outcome of interest.	
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	Medium	It was reported the test was conducted in a static system. In each test container, the solution was covered with a Teflon sheet. The test media preparation was not reported.	
	Metric 8: Consistency of Exposure Administration	Medium	The test was conducted in 100 mL of test solution in a static system kept at 20C with a 16L:8D photoperiod. Exposure concentrations appeared to be measured and were consistent for the study duration.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The DCHP was measured using HPLC. Measured concentrations can be found in Table 1 in the Japanese PDF.	
	Metric 10: Exposure Duration and Frequency	High	The study duration was reported to be 48h, which is typical of an acute toxicity test conducted with <i>Daphnia</i> .	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one test concentration was used for the definitive study.	
	Metric 12: Testing at or Below Solubility Limit	Medium	The test concentration slightly exceeded the water solubility limit, but a solvent was used to aid in dissolution. The concentration of the solvent was not reported.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	High	The <i>Daphnia magna</i> were reported to be from the National Institute for Environmental Studies. The organisms used in the test were <24h in age.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Adult <i>Daphnia</i> were reported to be acclimated to test conditions for nearly a month (Nov 17-Dec 13, 1999) prior to collecting <24h old young for the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There were 20 organisms per treatment and control with four replicates for a total of five organisms per test chamber.	
Domain 5: Outcome Assessment				
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<b>Study Citation:</b>	NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Immobilization			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803964			
Domain	Metric	Rating	Comments	
	Metric 16: Adequacy of Test Conditions	High	The organisms were kept at 20C with a 16L:8D photoperiod below an illumination of 800 lux. They were not fed for testing, but they were fed <i>Chlorella vulgaris</i> during acclimation at 0.2mg of organic content per organism per day. The ECHA states the water used was saltwater, but this is unlikely as these are freshwater organisms. Temperature, dissolved oxygen, and pH values were measured at the start and end of the test. Results for these water quality measurements are shown in Tables 5-7 in the Japanese pdf. Conditions were adequate. The number of organisms used per test container seemed adequate.	
	Metric 17: Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-immobilization.	
	Metric 18: Consistency of Outcome Assessment	Low	The methods for determining immobilization were not reported in the ECHA document and were not in English in the Japanese document. Thus, the methods for determining immobilization are unclear at this point. It appears they were assessed at 24 and 48h, but little else is known.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Daphnia were acclimated prior to the start of the test. Water quality conditions were adequate throughout the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	N/A	No immobilization was reported in the test concentration or in either of the controls, thus statistical analysis was not necessary.	
	Metric 22: Reporting of Data	High	Data for exposure related findings and control responses can be found in Table 2 of the Japanese PDF. Table 3 in the same PDF contains EiC50 values, and Table 4 has the NOEC values.	
	Metric 23: Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.	
Additional Comments:	This evaluation was for an acute toxicity test with <i>Daphnia magna</i> exposed to DCHP. Please note, this was a Japanese study in which a professional English translation was not available. This data was evaluated based off the tables in the Japanese PDF that were in English as well as an ECHA document for this particular study. Some metrics may have different ratings should a professionally translated English document become available.This evaluation is for the definitive test.			

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



<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803962			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The DCHP was identified by CASRN.
	Metric 2:	Test Substance Source	Low	It was not clear from the Japanese pdf if a source of the chemical was reported, or if the test substance was analytically verified. These two items were not reported in the ECHA document.
	Metric 3:	Test Substance Purity	High	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A pure control and a solvent control were used in this test.
	Metric 5:	Negative Control Response	Medium	In the negative control at day eight there was 20% mortality. It remained this percentage for the rest of the test through 21 days. The solvent control had zero mortalities for the duration of the test. According to OECD Guideline 211, a test is still valid if mortality of the parental <i>Daphnia</i> does not exceed 20% in the controls at the end of the test.
	Metric 6:	Randomized Allocation	Low	
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported the test was conducted in a semi-static system. The test solution was exchanged by whole volume every 24 hours. In each test container, the solution was covered with a Teflon sheet. The test media preparation was not reported, but the test concentrations were measured.
	Metric 8:	Consistency of Exposure Administration	Medium	
	Metric 9:	Measurement of Test Substance Concentration	Medium	The DCHP was measured using HPLC. Measured concentrations can be found in Table 1-1 in the Japanese pdf. Table 1-2 shows measured concentrations as a percentage of the nominal values.
	Metric 10:	Exposure Duration and Frequency	High	This <i>Daphnia magna</i> reproduction test ran 21 days. This is adequate per OECD Guideline 211.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were five DCHP test concentrations and two control groups used in this study. The first four test concentrations were close together and response effects were consistent across those (hardly any mortality). The last and highest test concentration was well above the one before it, and significant effects were seen with that one. An LC50 was calculated but it could have been more accurate had all five test concentrations been more evenly spaced.
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<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803962			
Domain	Metric	Rating	Comments	
	Metric 12:	Testing at or Below Solubility Limit	Medium	Only the highest test concentration was slightly above the water solubility limit. A solvent was used to aid in dissolution. The ECHA document mentioned a 3.2 vehicle constant of 100 mg/L dimethylformamide.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The <i>Daphnia magna</i> were reported to be from the National Institute for Environmental Studies. The organisms used in the test were less than 24 hours in age.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Adult <i>Daphnia</i> were reported to be acclimated to test conditions for approximately three weeks (Jan 19-Feb 7, 2000) prior to collecting <24h old young for the start of the test.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There was one <i>Daphnia magna</i> per test concentration container. There were ten containers per test concentration group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The organisms were kept at 20C with a 16L:8D photoperiod below an illumination of 800 lux. During acclimation parent <i>Daphnia</i> were fed <i>Cholrella vulgaris</i> at 0.2 mg of organic content per organism per day. During the exposure <i>Daphnia</i> were fed <i>Cholrella vulgaris</i> at 0.15 mg of organic content per organism per day. The ECHA states the water used was saltwater, but this is unlikely as these are freshwater organisms. Temperature, dissolved oxygen, pH, and total hardness were measured throughout the 21-day test. Results for these water quality parameters are shown in Tables 8-11 in the Japanese pdf. These conditions were adequate and fairly consistent throughout the test. One <i>Daphnia</i> per test container was adequate for this reproduction study.
	Metric 17:	Outcome Assessment Methodology	High	Mortality was indicated as one of the exposure effects assessed.
	Metric 18:	Consistency of Outcome Assessment	Low	The ECHA document did not indicate how mortality was assessed. Table 2-1 in the Japanese pdf shows the cumulative number of dead <i>Daphnia</i> per day for the duration of the test for each test concentration. The methods describing assessment of mortality are not in English in the Japanese pdf. If a professionally translated version becomes available this rating could change.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	<i>Daphnia</i> were acclimated prior to the start of the test. Water quality conditions were adequate throughout the exposure.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences among groups in animal attrition or health outcomes unrelated to exposure. However, there was 20% mortality in the negative control group.
Domain 7: Data Presentation and Analysis				
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<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Mortality			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803962			
Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	Low	Statistical analysis was not described in the ECHA document, and there were no methods described in English in the Japanese pdf. Table 5 in the Japanese pdf gives the calculated LC50 value, and it is shown that the Binomial statistical method was used. If a professionally translated version becomes available this rating could change.
	Metric 22:	Reporting of Data	High	The cumulative number of dead parental daphnia for each treatment concentration is shown in Table 2-1 and Figure 1 in the Japanese pdf. The percent mortality is shown in Table 2-2 and the LC50 value is reported in Table 5 of that same pdf.
	Metric 23:	Explanation of Unexpected Outcomes	High	Confidence limits (50%) were included in Table 5.
Additional Comments:	This evaluation is for mortality assessment of <i>Daphnia magna</i> during a 21-d reproduction test. There is some question as to the spacing of test concentrations used. And the 20% mortality in the negative control for over half of the test raises some concern.			

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

<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).		
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days		
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)		
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile		
<b>Health Outcome:</b>	Reproductive/Teratogenic		
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)		
<b>HERO ID:</b>	11803962		
Domain	Metric	Rating	Comments
Domain 1: Test Substance			
	Metric 1: Test Substance Identity	High	The DCHP was identified by CASRN.
	Metric 2: Test Substance Source	Low	It was not clear from the Japanese pdf if a source of the chemical was reported, or if the test substance was analytically verified. These two items were not reported in the ECHA document.
	Metric 3: Test Substance Purity	High	From the Japanese pdf, it appears the purity was 99.9%.
Domain 2: Test Design			
	Metric 4: Negative Controls	High	A pure control and a solvent control were used in this test.
	Metric 5: Negative Control Response	High	The time to first brood production was adequate and consistent in both control groups. Within both control groups the cumulative numbers of juveniles produced per adult seemed adequate and fairly consistent. Overall, more juveniles were produced in the solvent control than in the negative control.
	Metric 6: Randomized Allocation	Low	It was not reported how the organisms were allocated.
Domain 3: Exposure Characterization			
	Metric 7: Experimental System/Test Media Preparation	Medium	It was reported the test was conducted in a semi-static system. The test solution was exchanged by whole volume every 24 hours. In each test container, the solution was covered with a Teflon sheet. The test media preparation was not reported, but test concentrations were measured.
	Metric 8: Consistency of Exposure Administration	Medium	The test system was kept at 20C with a 16L:8D photoperiod. Each test <i>Daphnia</i> were fed the same amount per day of <i>Chlorella vulgaris</i> (0.15 mgC). Each test container had 80mL of test solution. Test concentrations were measured throughout the test. Some measured test concentrations exceeded +/-20% of nominal values.
	Metric 9: Measurement of Test Substance Concentration	Medium	The DCHP was measured using HPLC. Measured concentrations can be found in Table 1-1 in the Japanese pdf. Table 1-2 shows measured concentrations as a percentage of the nominal values.
	Metric 10: Exposure Duration and Frequency	High	This <i>Daphnia magna</i> reproduction test ran 21 days. This is adequate per OECD Guideline 211.
	Metric 11: Number of Exposure Groups/Spacing of Exposure Levels	High	There were five DCHP test concentrations and two control groups used in this study. Significant effects occurred in the second highest test concentration for the number of juveniles produced. A NOEC and LOEC were calculated from these results.
	Metric 12: Testing at or Below Solubility Limit	Medium	Only the highest test concentration was slightly above the water solubility limit. A solvent was used to aid in dissolution. The ECHA document mentioned a 3.2 vehicle constant of 100 mg/L dimethylformamide.
Domain 4: Test Organism			
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<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803962			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	High	The <i>Daphnia magna</i> were reported to be from the National Institute for Environmental Studies. The organisms used in the test were less than 24 hours in age.	
	Metric 14: Acclimatization and Pretreatment Conditions	High	Adult <i>Daphnia</i> were reported to be acclimated to test conditions for approximately three weeks (Jan 19-Feb 7, 2000) prior to collecting <24h old young for the start of the test.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	There was one <i>Daphnia magna</i> per test concentration container. There were ten containers per test concentration group.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	The organisms were kept at 20C with a 16L:8D photoperiod below an illumination of 800 lux. During acclimation parent <i>Daphnia</i> were fed <i>Cholrella vulgaris</i> at 0.2 mg of organic content per organism per day. During the exposure, <i>Daphnia</i> were fed <i>Cholrella vulgaris</i> at 0.15 mg of organic content per organism per day. The ECHA states the water used was saltwater, but this is unlikely as these are freshwater organisms. Temperature, dissolved oxygen, pH, and total hardness were measured throughout the 21-day test. Results for these water quality parameters are shown in Tables 8-11 in the Japanese pdf. These conditions were adequate and fairly consistent throughout the test. One <i>Daphnia</i> per test container was adequate for this reproduction study.	
	Metric 17: Outcome Assessment Methodology	High	The number of days to first brood production and the number of juveniles produced per adult were indicated as the two exposure effects assessed.	
	Metric 18: Consistency of Outcome Assessment	Low	The ECHA document did not describe methods used for assessing the reproductive outcomes. And there were no details on this in English in the Japanese pdf. From Table 3 and 4 (Japanese pdf) it appears that the reproductive effects were assessed daily. If a professionally translated version becomes available, this rating could change.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	Daphnia were acclimated prior to the start of the test. Water quality conditions were adequate throughout the exposure.	
	Metric 20: Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences among groups in animal attrition or health outcomes unrelated to exposure. However, there was 20% mortality in the negative control group.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Statistical analysis was not described in the ECHA document and there were no methods described in English in the Japanese pdf. Table 6 in the Japanese pdf gives the calculated EC50 value (inhibition of reproduction), and it is shown that the Logit statistical method was used. Table 7 in the Japanese pdf indicates that Dunnett’s multi-comparison test was used to assess data on numbers of juveniles produced per adult. If a professionally translated version becomes available this rating could change.	
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<b>Study Citation:</b>	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for <i>Daphnia magna</i> (translation).			
<b>Duration:</b>	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile			
<b>Health Outcome:</b>	Reproductive/Teratogenic			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803962			
Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	High	In the Japanese pdf, Tables 3, 4, 6, and 7 show reproductive effects results. Figure 2 also shows reproductive effects data. Table 6 gives the calculated EC50 value for inhibition of reproduction.
	Metric 23:	Explanation of Unexpected Outcomes	High	Table 7 (Japanese pdf) includes standard deviations.
Additional Comments:	This evaluation is for the reproductive assessment of <i>Daphnia magna</i> during a 21-d reproduction test. There was 20% mortality in the negative control for over half of the test, which raises some concern.			
<b>Overall Quality Determination</b>			<b>High</b>	

<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical was identified by correct nomenclature and chemical structure. CASRN was not reported. DCHP was purchased from Fluka Chem. Co. Purity was not reported.
	Metric 2:	Test Substance Source	High	
	Metric 3:	Test Substance Purity	Low	
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	Biological responses of controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors reported that the phthalate stocks were separately dissolved in acetone and diluted with M-199 (hemocyte-culture medium) to a concentration of 1000 mg/ml. They also reported the final concentration once the phthalates were added to the hemocyte suspension. However, the authors did not report the acetone concentration employed. The exposure was consistent.
	Metric 8:	Consistency of Exposure	High	
	Metric 9:	Administration	Low	
	Metric 10:	Measurement of Test Substance Concentration	High	Exposure concentrations were not reported/measured.
	Metric 11:	Exposure Duration and Frequency	High	An appropriate exposure time was used.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentrations (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	
The test substance was below the solubility limit as reported. But the authors did not report the concentration of acetone used to dissolve the phthalate and did not measure the phthalate concentration.				
Domain 4: Test Organism				
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
	Metric 13: Test Organism Characteristics	Medium	Test organisms (freshwater prawn) were obtained from a market and hemolymph drawn from these test organisms was then used to isolate the hemocytes employed in the in vitro exposure and assays. Information on the size/age of prawns was not provided.	
	Metric 14: Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 C for 3 days prior to experiments, and stocking densities were maintained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect immune function (i.e., stress can modulate immune responses (cell-mediated and humoral). The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.	
	Metric 15: Number of Organisms and Replicates per Group	Medium	This was an in vitro study to determine impacts on nonspecific cell-mediated immune defense responses. The number of organisms used (5-10) to collect a diverse pool of hemocytes, number of cells and cell suspensions, number of well-plates, and number of replicate fields for microscopic counts were sufficient.	
Domain 5: Outcome Assessment				
	Metric 16: Adequacy of Test Conditions	High	This was an in vitro exposure study to determine impacts on nonspecific cell-mediated immune defense responses. In vitro exposure conditions were adequate.	
	Metric 17: Outcome Assessment Methodology	High	Outcome assessment methodologies [determination of hemocytic adhesion and pseudopodia formation assay, the phenoloxidase activity assay, and the nitroblue tetrazolium assay] were reported and appropriate for the outcomes of interest [phagocytosis and encapsulation activity, pathogen recognition, and superoxide activity as a measure of microbicidal activity].	
	Metric 18: Consistency of Outcome Assessment	High	Details of the immune function in vitro assays were reported and assessed consistently across groups.	
Domain 6: Confounding / Variable Control				
	Metric 19: Confounding Variables in Test Design and Procedures	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared in adequate medium conditions and treated consistently across treatment groups.	
	Metric 20: Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.	
Domain 7: Data Presentation and Analysis				
	Metric 21: Statistical Methods	Low	Statistical analysis was reported but not explained in detail.	
	Metric 22: Reporting of Data	High	Data were reported for each assay and for all treatment groups per assay. The summary of the effects in Table 1 reported the inhibition or enhancement concentration for each assay.	
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)
<b>HERO ID:</b>	789598

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	Authors reported the standard error of the mean for the nonspecific immune response data: hemocyte adhesion and pseudopodia formation, phenoloxidase activity, and superoxide production (Figures 2 and 3).

Additional Comments: This study was an in vitro experiment where hemocytes (immune cells) isolated from the hemolymph of 5 to 10 giant freshwater prawn (*Macrobrachium rosenbergii*) were exposed to BBP, DBP, DEHP, or DCHP. Endpoints encompassed nonspecific cell-mediated immune function assays as well as hemocyte viability assays: (1) Nonspecific cell-mediated immune defense response assays included determination of hemocytic adhesion and pseudopodia formation (a measure of the initial procedures of either phagocytosis or encapsulation), phenoloxidase activity assay (a measure of pathogen recognition and defense functions), and nitroblue tetrazolium solution (NBT) assay to determine superoxide production (a measure of highly microbicidal activity); (2) Hemocyte toxicity/viability assays included detection of cell death via necrosis, detection of cell death via apoptosis, and impacts on cellular morphology assessed by microscopy. This form was used to evaluate impacts to the nonspecific cell-mediated immune defense responses due to DCHP.

## Overall Quality Determination

## Medium

<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical was identified by correct nomenclature and chemical structure. CASRN was not reported.
	Metric 2:	Test Substance Source	High	The source of DCHP was Aldrich Chem. Co.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used.
	Metric 5:	Negative Control Response	High	Biological responses of controls were appropriate.
	Metric 6:	Randomized Allocation	Low	Organisms were purchased from local prawn farms on separate days. The hemocytes isolated from 5 or 10 prawns were used for the assays, but the authors did not specifically mention random allocation of the hemocytes from different prawns for the various assays.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors reported that the phthalate stocks were separately dissolved in acetone and diluted with M-199 (hemocyte-culture medium) to a concentration of 1000 mg/ml. They also reported the final concentration once the phthalates were added to the hemocyte suspension. However, the authors did not report the acetone concentration employed.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported/measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was appropriate to assess cell death (necrosis and apoptosis) and cell morphology impacts in the hemocytes.
	Metric 11:	Number of Exposure Groups/Spacing of Exposure Levels	Medium	In Table 1, the authors reported treatment with different concentrations (25, 50 and 100 mg/ml) of PAEs. However, there is limited information in the text, and it is unclear how each concentration was achieved and if all three concentrations were included in all assays or only some of the assays.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The test substance was below the solubility limit as reported. But the authors did not report the concentration of acetone used to dissolve the phthalate and did not measure the phthalate concentration.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organisms (freshwater prawn) were obtained from a market and hemolymph drawn from these test organisms was then used to isolate the hemocytes employed in the in vitro exposure and assays. Information on the size/age of prawns was not provided.

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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	789598			
Domain	Metric	Rating	Comments	
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Test organisms (freshwater prawn) were acclimated in fresh pond water in 120 L plastic containers at 30 8C for three days prior to experiments, and stocking densities were maintained at 20 prawns per container. However, there was no indication of the health or stress status of the test organisms, which can affect the immune system. The exposure to phthalates was in vitro using hemocytes (immune cells) isolated from prawns.
	Metric 15:	Number of Organisms and Replicates per Group	Low	This was an in vitro study to determine impacts on hemocytes (immune cells) measured by cell death (necrosis and apoptosis) and cell morphology. The number of organisms used (5-10) to collect a diverse pool of hemocytes and cell suspensions was reported. However, authors offered insufficient details on, for example, the number of replicates examined by gel electrophoresis or the number of sections and replicate fields for the electron microscopy assessment.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	This was an in vitro exposure study to determine impacts on hemocytes measured by cell death (necrosis and apoptosis) and cell morphology. In vitro exposure conditions were adequate.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methodologies using hemocytes [the annexin assay, gel electrophoresis, transmission electron microscopy] were reported and appropriate for the outcomes of interest in hemocytes [cell death by necrosis and apoptosis and cell morphology changes].
	Metric 18:	Consistency of Outcome Assessment	High	Details of the in vitro assays to examine hemocyte toxicity/viability were reported and assessed consistently across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study was an in vitro exposure. As described, hemocyte suspensions were prepared in adequate medium conditions and treated consistently.
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was reported but not explained in detail.
	Metric 22:	Reporting of Data	High	Data were reported for each assay and for all treatment groups per assay. The summary of the effects in Table 1 reported the inhibition or enhancement concentration for each assay.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Authors did not report SE, SD, CI or such variability details for the cell death (necrosis and apoptosis) and cell morphology data.
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<b>Study Citation:</b>	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, <i>Macrobrachium rosenbergii</i> . Aquatic Toxicology 64(1):25-37.
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Mechanistic-Cell signaling/function
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)
<b>HERO ID:</b>	789598

Domain	Metric	Rating	Comments
Additional Comments:	This study was an in vitro experiment where hemocytes (immune cells) isolated from the hemolymph of 5 to 10 giant freshwater prawn ( <i>Macrobrachium rosenbergii</i> ) were exposed to BBP, DBP, DEHP, or DCHP. Endpoints encompassed nonspecific cell-mediated immune function assays as well as hemocyte viability assays: (1) Nonspecific cell-mediated immune defense response assays included determination of hemocytic adhesion and pseudopodia formation (a measure of the initial procedures of either phagocytosis or encapsulation), phenoloxidase activity assay (a measure of pathogen recognition and defense functions), and nitroblue tetrazolium solution (NBT) assay to determine superoxide production (a measure of highly microbicidal activity); (2) Hemocyte toxicity/viability assays included detection of cell death via necrosis, detection of cell death via apoptosis, and impacts on cellular morphology assessed by microscopy. This form was used to evaluate hemocyte toxicity/viability due to DCHP.		

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

<b>Study Citation:</b>	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae ( <i>Selenastrum capricornutum</i> ) (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803966			
Domain	Metric	Rating	Comments	
Domain 1: Test Substance				
	Metric 1: Test Substance Identity	High	The DCHO was identified by CASRN.	
	Metric 2: Test Substance Source	Low	The source of the test substance was not reported, nor was it reported if it was analytically verified by the performing laboratory.	
	Metric 3: Test Substance Purity	High	It appears the purity was 99.9% (seen in the Japanese pdf).	
Domain 2: Test Design				
	Metric 4: Negative Controls	High	It was reported that there was a culture media only control as well as a solvent control that used dimethylformamide.	
	Metric 5: Negative Control Response	High	The negative control response and the solvent control response were reported in Table 2 and Table 3 and were adequate for the outcome of interest.	
	Metric 6: Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.	
Domain 3: Exposure Characterization				
	Metric 7: Experimental System/Test Media Preparation	High	The test substance was prepared by weighing 100mg of DCHP and dissolving it into 200mg of dimethylformamide. A dispersing agent (4800mg HCO-40) was added and mixed fully. This mixture was brought to 100mL by using a volumetric flask using culture media. An aliquot of test media was added to each test chamber, and the appropriate amount of vehicle was added along with the test solution. The test system was reported to be static in the ECHA.	
	Metric 8: Consistency of Exposure Administration	Low	The ECHA did not report the test chamber dimension or the test volume used in the study. Little information was provided on the test conditions. This metric may change should a professionally translated English version become available.	
	Metric 9: Measurement of Test Substance Concentration	Medium	The test concentrations were measured using HPLC and were reported in Table 1 of the Japanese PDF.	
	Metric 10: Exposure Duration and Frequency	High	The exposure duration was reported to be 72h. This was adequate for the outcome of interest.	
	Metric 11: Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure concentration in this test (2.00mg/L DCHP).	
	Metric 12: Testing at or Below Solubility Limit	High	The test concentration was above the water solubility limit, but a solvent was used to aid in dissolution. The solvent control response was adequate.	
Domain 4: Test Organism				
	Metric 13: Test Organism Characteristics	Low	It was unclear from the ECHA what the source of the algae was for this study. It is also unclear if the algae was in the logarithmic growth phase.	

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<b>Study Citation:</b>	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae ( <i>Selenastrum capricornutum</i> ) (translation).			
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)			
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported			
<b>Health Outcome:</b>	Development/Growth			
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)			
<b>HERO ID:</b>	11803966			
Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Little information was provided in the ECHA on the test conditions other than test temperature and pH. The culture conditions were not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The initial algal cell concentration appeared to be 10,000 cells/mL as reported in Table 2 of the Japanese PDF. It was reported in the ECHA that there were three replicates for the test concentration and six replicates for the controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Little information on the test conditions and culturing conditions was provided in the ECHA. Only test temperature and pH were reported in the Japanese PDF. This metric rating could change should a English version become available.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-development/growth in terms of growth inhibition of algal cells for 72h.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were limited. The ECHA did not provide much information regarding the outcome assessment process. It is possible the Japanese PDF has more information regarding this, but an English translation would be needed to confirm this.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported in the Japanese PDF under Table 4. Yukes Statlight #3 software was used to conduct statistical analyses.
	Metric 22:	Reporting of Data	High	Data for the control and exposure responses is reported in Tables 2 and 3 of the Japanese PDF and appears adequate for the outcome of interest. EC50 and NOEC values are reported in Table 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	There did not appear to be any unexpected outcomes. Average SD is reported in Tables 2 and 3.
Additional Comments:	This evaluation was on the effect of DCHP on growth inhibition of the algae <i>Selenastrum capricornutum</i> . Please note, two documents were used for this evaluation. One was an ECHA PDF and the other was the original document in Japanese that contained some tables and figures in English. Should a professionally translated document in English become available, some metric ratings may change.			

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

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<b>Study Citation:</b>	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae ( <i>Selenastrum capricornutum</i> ) (translation).
<b>Duration:</b>	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
<b>Exposure Route, Media, Path:</b>	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
<b>Taxa, Species, Age:</b>	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported
<b>Health Outcome:</b>	Development/Growth
<b>Chemical:</b>	Dicyclohexyl phthalate (DCHP)
<b>HERO ID:</b>	11803966

Domain	Metric	Rating	Comments
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