

December 2024 Office of Chemical Safety and Pollution Prevention

Draft 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 Draft Risk Evaluation**

CASRN: 84-61-7



December 2024

This supplemental file contains information regarding the data quality evaluation results relevant to the analysis of environmental hazard for the *Draft Environmental Hazard Assessment for 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 performs data quality evaluation as a part of 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'). The systematic review steps are further described in the *Draft Risk Evaluation for Dicyclohexyl Phthalate (DCHP)* – *Systematic Review Protocol*.

Different data quality evaluation forms were used depending on the organism as described in the PECO (Population, Exposure, Comparator or Scenario, and Outcomes) 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, 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

Dicyclohexyl Phthalate

HERO II	)	Reference	Page
Dic	yclohexyl Phthal	ate	
Hab	oitat: Aquatic (fresh	water)	
Т	axa: Vertebrates		
	Oryzias latipes		
11803931		NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka (Oryzias latipes) (translation).	4
	Silurana tropicalis		
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 Silurana tropicalis larvae. Environmental Toxicology and Chemistry 35(10):2511–2522.	7
T	axa: Invertebrates		
	Daphnia magna		
11803964		NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on Daphnia magna (translation).	13
11803962		NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for Daphnia magna (translation).	17
	Macrobrachium rosenbergii		
789598		Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.	23
Т	axa: Plants (Non-vascul	ar)	
	Selenastrum capricornutum		
11803966		NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae (Selenastrum capricornutum) (translation).	29

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical:	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka (Oryzias latipes) (translation). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Fish; <i>Oryzias latipes</i> ; Juvenile Mortality Dicyclohexyl phthalate (DCHP)						
Domain	11005751	Matria	Dating	Commonto			
Domain 1: Test Substan	Metric Kaung Comments						
Domain 1. Test Substant	Metric 1.	Test Substance Identity	High	The CASEN was included in the Japanese ndf			
	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 <sup>.</sup> Exposure Ch	aracterization						
Domain 5. Exposure Ch	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 doc- ument mentions that the 2mg/L test concentration was the highest dispersible concentra- tion 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 concentra- tion (2 mg/L) shown in the results tables. In the executive summary of the ECHA doc- ument 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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Dicyclohexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path:	NITE, (2000). Acute toxicity study of dicyclohexyl phthalate on Japanese medaka (Oryzias latipes) (translation). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)						
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vertebrate; F Mortality Dicyclohexy 11803931	ish; <i>Oryzias latipes</i> ; Juvenile l phthalate (DCHP)					
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 fur- ther 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 Organis	m						
Domain 1. Test organis	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 be- fore 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 Ass	sessment						
Domain 5. Outcome As	Metric 16:	Adequacy of Test Conditions	High	Fish were reported to be healthy, and the mortality rate was below 5% during the accli- mation 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 ade- quately described in the ECHA document.			
	Metric 18:	Consistency of Outcome Assessment	High	The ECHA document reported that mortality was assessed across all exposure concen- trations every 24 hours.			
Domain 6: Confounding	g / Variable Cor Metric 19:	trol 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.			

Environmental Hazard Evaluation

HERO ID: 11803931 Table: 1 of 1

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vs in animal					
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os in animal					
<b>5</b> 61 (11					
5% confidence icentration posure con-					
ilated LC50 the ECHA					
ity were not ortalities ob-					
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.					

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Dicyclohexyl Phthalate

Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	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 Silurana tropicalis larvae. Environmental Toxicology and Chemistry 35(10):2511–2522. Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route) Vertebrate; Amphibian; <i>Silurana tropicalis</i> ; Larvae Mortality Dicyclohexyl phthalate (DCHP) 3230411						
Domain		Metric	Rating	Comments			
Domain 1: Test Substance	ce						
	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 2. Euroques Ch	anastanization						
Domain 5: Exposure Cha	Matria 7	E	T				
	Metric 7:	Preparation	Low	mental 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	High	Study authors reported using GC-MS to measure the test substances.			
	Metric 10:	Concentration 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 Organist	n						
C	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	Low	It was not reported if the organisms were acclimated.			
	Metric 15:	Conditions 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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Dicyclohexyl Phthalate

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Study Citation:	Mathieu-Der	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 desters in Silurana tropicalis larvae. Environmental Toxicology and Chemistry 35(10):2511–2522							
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)								
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)								
Media, Path:									
Taxa, Species, Age:	Vertebrate; Amphibian; Silurana tropicalis; Larvae								
Health Outcome:	Mortality								
Chemical:	Dicyclohexyl phthalate (DCHP)								
HERO ID:	3230411								
Domain		Metric	Rating	Comments					
Domain 5: Outcome As	sessment								
	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 load- ing 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	High	Details of the outcome assessment protocol were reported, and outcomes were assessed					
		Assessment		consistently across study groups. Embryos were monitored daily for mortalities, and dead embryos removed at this point.					
Domain 6: Confounding	y / Variable Co	ntrol							
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.					
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.					
Domain 7: Data Present	tation and Anal	vsis							
	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 supplementa	of the evaluation was on the effect of DCH l material that was not included with the D	IP on embry istiller down	o mortality in S. tropicalis. Mortality was selected as the outcome of interest. There is load. This contains more data regarding the mechanistic outcomes in the paper.					
<b>Overall Quali</b>	ty Detern	nination	High						

Dicyclohexyl Phthalate

Study Citation:	Mathieu-Der	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								
Duration: Exposure Route, Media, Path:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)									
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Silurana tropicalis; Larvae								
Health Outcome:	Developmen	t/Growth								
Chemical:	Dicyclohexy	l phthalate (DCHP)								
HERO ID:	3230411									
Domain		Metric	Rating	Comments						
Domain 1: Test Substand	ce									
	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 2. Expedience Ch	anastanization									
Domain 5: Exposure Ch	Metric 7	Experimental System/Test Media	Low	Limited datails were provided on the preparation of the test media. The type of experi						
	Wieure 7.	Preparation	Low	mental container material used (glass vs plastic) was not reported.						
	Metric 8:	Consistency of Exposure	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	High	Study authors reported using GC-MS to measure the test substances.						
	Metric 10:	Concentration 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/	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: Tast Organise	m									
Domain 4: Test Organisi	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	Low	It was not reported if the organisms were acclimated.						
	Metric 15:	Conditions Number of Organisms and	Medium	The number of organisms used in each test concentration is reported in Tables 1 and 4.						
		Replicates per Group								

Domain 5: Outcome Assessment

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HERO ID: 3230411 Table: 2 of 3

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Study Citation:	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 Silurana tropicalis larvae. Environmental Toxicology and Chemistry 35(10):2511–2522.								
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)								
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)								
Faxa, Species, Age:	Vertebrate; A	Amphibian; Silurana tropicalis; Larvae							
Health Outcome:	Development/Growth Dicyclohexyl phthalate (DCHP)								
Chemical:									
HERO ID:	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 load- ing 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	r / Variable Co	ntrol							
Domain 0. Comounding	Metric 19.	Confounding Variables in Test	Low	The study did not provide enough information to allow a comparison of environmental					
	infeate 19.	Design and Procedures	Low	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 Present	ation and Anal	vsis							
Johnani 7. Duta i resent	Metric 21	Statistical Methods	High	Statistical methods were described in detail in the "Data Analysis" section of the naper					
	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 Ta- bles 1 and 4.					
Additional Comments:	: This portion of the evaluation was on the effect of DCHP on embryo development and malformations in S. tropicalis. 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

Dicyclohexyl Phthalate

erall Duration uatic (freshwa tebrate; Ampl chanistic-Bio: yclohexyl pht 0411 tric 1: Te tric 2: Te tric 2: Te tric 3: Te	: 0 - 4 days (0-96h); Exposure Duration tter); Water; Not determined by study hibian; <i>Silurana tropicalis</i> ; Larvae markers (exposure and effect)-Cell sign thalate (DCHP) Metric est Substance Identity est Substance Purity	on: 0 - 4 days authors (i.e., - maling/function Rating High Low High	(0-96h) chemical of interest in exposure water, but unable to determine exact uptake route) on <u>Comments</u> The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tebrate; Ampl chanistic-Bio yclohexyl pht 0411 tric 1: Ta tric 2: Ta tric 3: Ta	hibian; <i>Silurana tropicalis</i> ; Larvae markers (exposure and effect)-Cell sig thalate (DCHP) <u>Metric</u> est Substance Identity est Substance Source est Substance Purity	naling/functi Rating High Low High	on Comments The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
chanistic-Bio yclohexyl pht 0411 tric 1: Ta tric 2: Ta tric 3: Ta tric 4: N	markers (exposure and effect)-Cell sig thalate (DCHP) Metric est Substance Identity est Substance Source est Substance Purity	Rating/functi Rating High Low High	The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
yclohexyl pht 0411 tric 1: Ta tric 2: Ta tric 3: Ta tric 4: N	thalate (DCHP) Metric est Substance Identity est Substance Source est Substance Purity	Rating High Low High	Comments The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
10411       tric 1:     Te       tric 2:     Te       tric 3:     Te       tric 4:     N	Metric est Substance Identity est Substance Source est Substance Purity	Rating High Low High	Comments The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tric 1: To tric 2: To tric 3: To tric 4: N	Metric est Substance Identity est Substance Source est Substance Purity	Rating High Low High	Comments The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tric 1: To tric 2: To tric 3: To tric 4: N	est Substance Identity est Substance Source est Substance Purity	High Low High	The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tric 1: Ta tric 2: Ta tric 3: Ta tric 4: N	est Substance Identity est Substance Source est Substance Purity	High Low High	The DCHP was identified by CAS no. The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tric 2: To tric 3: To tric 4: N	est Substance Source	Low High	The DCHP was reported to be from Arcos Organics, but it was not reported if it was analytically verified.
tric 3: To	est Substance Purity	High	
tric 4· N		0	The purity was reported to be 99%.
tric 4 · N			
une <del>4</del> . 19	egative Controls	High	Study authors reported the use of a water control and a solvent control (0.82% DMSO).
tric 5: N	egative 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.
tric 6: R	andomized Allocation	Low	It was not reported how the embryos were allocated into study groups.
erization			
tric 7: E	xperimental System/Test Media	Low	Limited details were provided on the preparation of the test media.
tric 8: C A	onsistency of Exposure dministration	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.
tric 9: M	leasurement of Test Substance	High	Study authors reported using GC-MS to measure the test substances.
tric 10: C	oncentration xposure Duration and Frequency	Medium	The exposure was 72h, and study authors reported this was shorter than the typical 96h test.
tric 11: N	umber of Exposure Groups/	High	There were five exposure groups and the spacing was adequate to observe a response.
tric 12: To	esting 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.
tric 13: To	est 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.
tric 14: A	cclimatization and Pretreatment	Low	It was not reported if the organisms were acclimated.
tric 15: C R	onditions umber of Organisms and eplicates per Group	Medium	The number of organisms used in each test concentration is reported in Tables 1 and 4.
tri tri tri tri tri tri tri tri tri tri	ic 4. $N$ $ic 5$ : $N$ $ic 5$ : $N$ $ic 6$ : $R$ $ic 7$ : $E$ $ic 7$ : $E$ $ic 8$ : $C$ $ic 9$ : $N$ $ic 10$ : $E$ $ic 11$ : $N$ $ic 12$ : $T$ $ic 13$ : $T$ $ic 14$ : $A$ $ic 15$ : $N$ $nt$ $nt$	<ul> <li>ic 4. Regative Controls</li> <li>ic 5: Negative Control Response</li> <li>ic 6: Randomized Allocation</li> <li>ization</li> <li>ic 7: Experimental System/Test Media Preparation</li> <li>ic 8: Consistency of Exposure Administration</li> <li>ic 9: Measurement of Test Substance Concentration</li> <li>ic 10: Exposure Duration and Frequency</li> <li>ic 11: Number of Exposure Groups/ Spacing of Exposure Levels</li> <li>ic 12: Testing at or Below Solubility Limit</li> <li>ic 13: Test Organism Characteristics</li> <li>ic 14: Acclimatization and Pretreatment Conditions</li> <li>ic 15: Number of Organisms and Replicates per Group</li> </ul>	ic 4.       Negative Controls       High         ic 5:       Negative Control Response       Medium         ic 6:       Randomized Allocation       Low         ization       ic 7:       Experimental System/Test Media       Low         ization       ic 8:       Consistency of Exposure       Low         ic 8:       Consistency of Exposure       Low         Administration       ic 9:       Measurement of Test Substance       High         ic 10:       Exposure Duration and Frequency       Medium         ic 11:       Number of Exposure Groups/       High         Spacing of Exposure Levels       High         ic 12:       Test Organism Characteristics       High         ic 13:       Test Organism Characteristics       High         ic 14:       Acclimatization and Pretreatment       Low         Conditions       Ic 15:       Number of Organisms and       Medium         ic 15:       Number of Organisms and       Medium         nt       Replicates per Group       Medium

**December 2024** Environmental Hazard Evaluation

Dicyclohexyl Phthalate

	continued from previous page									
Study Citation:	Mathieu-Der diesters in Si	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 Silurana tropicalis larvae. Environmental Toxicology and Chemistry 35(10):2511–2522.								
Duration:	Overall Dura	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)								
Exposure Route,	Aquatic (fres	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)								
Media, Path:										
Taxa, Species, Age:	Vertebrate; A	Vertebrate; Amphibian; Silurana tropicalis; Larvae								
Health Outcome:	Mechanistic-	Mechanistic-Biomarkers (exposure and effect)-Cell signaling/function								
Chemical:	Dicyclohexy	l phthalate (DCHP)								
HERO ID:	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	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 Present	ation and Anal	ysis								
	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.						
Additional Comments:	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.									

**Overall Quality Determination** 

High

Study Citation:NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on Daphnia magna (translation).Duration:Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)Exposure Route,Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determineMedia, Path:Output									
Taxa, Species, Age:	Invertebrate; Arthropods; <i>Daphnia magna</i> ; Juvenile Immobilization								
Health Outcome:									
Chemical:	Dicyclohexy	l phthalate (DCHP)							
HERO ID:	11803964								
Domain		Metric	Rating	Comments					
Domain 1: Test Substand	ce								
	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 deter- mined 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 Ch	aracterization								
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported the test was conducted in a static system. The solution in the test con- tainers 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	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 Daphnia.					
	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 Organis	m								
C	Metric 13:	Test Organism Characteristics	High	The Daphnia magna 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 Daphnia 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.					
		Conti	nued on next pa	ige					

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Dicyclohexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 11803964 Table: 1 of 2

		contin	ued from previ	ous page				
Study Citation: Duration: Exposure Route, Media. Path:	<ul> <li>NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on Daphnia magna (translation). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)</li> <li>Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route</li> </ul>							
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Immobilizati Dicyclohexy 11803964	Arthropods; <i>Daphnia magna</i> ; Juvenile ion l phthalate (DCHP)						
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 As	sessment							
	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 Chlorella vulgaris 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	g / Variable Co	ntrol						
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.				
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.				
Domain 7: Data Present	ation and Anal	ysis						
	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 evaluati translation w particular stu the prelimina	ion was for an acute toxicity test with Daphni vas not available. This data was evaluated ba udy. Some metrics may have different rating ary test.	a magna expose ased off the tabl s should a prof	ed to DCHP. Please note, this was a Japanese study in which a professional English les in the Japanese PDF that were in English as well an ECHA document for this essionally translated English document become available. This evaluation was for				

**Overall Quality Determination** 

Medium

Chemical: HERO ID:	Dicyclohexy 11803964	l phthalate (DCHP)		
Domain		Metric	Rating	Comments
Domain 1: Test Substa	ance		-	
	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 deter- mined 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 Desig	n			
	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			
Domain 5. Exposure v	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 Daphnia.
	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 Organ	nism			
	Metric 13:	Test Organism Characteristics	High	The Daphnia magna 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 Daphnia were reported to be acclimated to test conditions for nearly a month (No 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.

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

NITE, (2000). Acute inhibition test of dicyclohexyl phthalate on Daphnia magna (translation).

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

Invertebrate; Arthropods; Daphnia magna; Juvenile

Immobilization

HERO ID: 11803964 Table: 2 of 2

Dicyclohexyl Phthalate

**Study Citation:** 

**Exposure Route**,

**Health Outcome:** 

Media, Path: Taxa, Species, Age:

**Duration:** 

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Dicyclohexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 11803964 Table: 2 of 2

		contin	ued from previ	ious page
Study Citation: Duration: Exposure Route, Media Path:	NITE, (2000 Overall Dura Aquatic (frea	)). Acute inhibition test of dicyclohexyl phth ation: 0 - 4 days (0-96h); Exposure Duration shwater); Water; Not determined by study au	alate on Daphni : 0 - 4 days (0-9 thors (i.e., chen	a magna (translation). 96h) nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Invertebrate; Immobilizat Dicyclohexy 11803964	Arthropods; <i>Daphnia magna</i> ; Juvenile ion l phthalate (DCHP)		
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 Chlorella vulgaris during ac- climation 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. Re- sults 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 ad- equate.
	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 Co	ntrol		
	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 Present	ation and Anal	vsis		
	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 Daphnia magna 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

Study Citation: Duration: Exposure Route, Media, Path:	NITE, (2000) Overall Dura Aquatic (fres	). Dicyclohexyl phthalate: Reproduction ir tion: 11 - 21 days; Exposure Duration: 11 hwater); Water; Not determined by study a	nhibition test for I - 21 days authors (i.e., chen	Daphnia magna (translation). nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Mortality			
Chemical:	Dicyclohexy	l phthalate (DCHP)		
HERO ID:	11803962			
Domain		Metric	Rating	Comments
Domain 1: Test Substand	ce		TT' 1	
	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	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 Daphnia does not exceed 20% in the controls at the end of the test.
	Metric 6:	Randomized Allocation	Low	It was not reported how the organisms were allocated.
	,			
Domain 5: Exposure Ch	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 Teffon cheet. The test media preparation was not reported, but the 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 Daphnia were fed the same amount per day of Chlorella vulgaris (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 Daphnia magna reproduction test ran 21 days. This is adequate per OECD Guide- line 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 con- sistent 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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Environmental Hazard Evaluation

HERO ID: 11803962 Table: 1 of 2

		contil	nued from previ	ous page
Study Citation: Duration: Exposure Route, Media, Path:	NITE, (2000 Overall Dura Aquatic (free	). Dicyclohexyl phthalate: Reproduction in ation: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study a	hibition test for I - 21 days uthors (i.e., chen	Daphnia magna (translation). nical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Mortality			
Chemical: HERO ID:	Dicyclohexy 11803962	l phthalate (DCHP)		
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 Organi	sm			
	Metric 13:	Test Organism Characteristics	High	The Daphnia magna 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 Daphnia 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 Daphnia magna per test concentration container. There were ten contain- ers per test concentration group.
Domain 5: Outcome A	ssessment			
	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 Daphnia were fed Cholrella vulgaris at 0.2 mg of organic content per organism per day. During the exposure Daphnia were fed Cholrella vulgaris 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 Daphnia 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 Daphnia 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: Confoundin	σ / Variable Co	atrol		
2 smain 0. Comoundin	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 attri- tion or health outcomes unrelated to exposure. However, there was 20% mortality in the negative control group.

Continued on next page ...

Dicyclohexyl Phthalate

Dicyclohexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 11803962 Table: 1 of 2

		continu	ed from previ	ious page		
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	NITE, (2000 Overall Dura Aquatic (fre: Invertebrate; Mortality Dicyclohexy 11803962	<ul> <li>NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for Daphnia magna (translation).</li> <li>Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days</li> <li>Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)</li> <li>Invertebrate; Arthropods; <i>Daphnia magna</i>; Juvenile</li> <li>Mortality</li> <li>Dicyclohexyl phthalate (DCHP)</li> <li>11803962</li> </ul>				
Domain		Metric	Rating	Comments		
	Metric 21:	Statistical Methods	Low	Statistical analysis was not described in the ECHA document, and there were no meth- ods described in English in the Japanese pdf. Table 5 in the Japanese pdf gives the cal- culated 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:	ments: This evaluation is for mortality assessment of Daphnia magna 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.					
<b>Overall Qualit</b>	ty Detern	nination	Medium	L		

Environmental Hazard Evaluation

Study Citation: Duration: Exposure Route, Media, Path:	NITE, (2000 Overall Dura Aquatic (fres	). Dicyclohexyl phthalate: Reproduction in tion: 11 - 21 days; Exposure Duration: 11 shwater); Water; Not determined by study a	nhibition test - 21 days authors (i.e.,	for Daphnia magna (translation). chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate;	Arthropods; Daphnia magna; Juvenile		
Health Outcome:	Reproductive	e/Teratogenic		
Chemical:	Dicyclohexy	l phthalate (DCHP)		
HERO ID:	11803962			
Domain		Metric	Rating	Comments
Domain 1: Test Substan	ce			
	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 Ch	aracterization	E-manimum and all Constants /Track Marking	Madian	
	Metric 7:	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 con- centrations were measured.
	Metric 8:	Consistency of Exposure Administration	Medium	The test system was kept at 20C with a 16L:8D photoperiod. Each test Daphnia were fed the same amount per day of Chlorella vulgaris (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 Daphnia magna reproduction test ran 21 days. This is adequate per OECD Guide- line 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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Dicyclohexyl Phthalate

Environmental Hazard Evaluation

HERO ID: 11803962 Table: 2 of 2

		cont	inued from p	revious page
Study Citation: Duration: Exposure Route, Madia Dath:	NITE, (2000 Overall Dura Aquatic (free	)). Dicyclohexyl phthalate: Reproduction ation: 11 - 21 days; Exposure Duration: 1 shwater); Water; Not determined by study	inhibition test 1 - 21 days authors (i.e.,	for Daphnia magna (translation). chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age: Health Outcome: Chemical: HFRO ID:	Invertebrate; Reproductiv Dicyclohexy	; Arthropods; <i>Daphnia magna</i> ; Juvenile e/Teratogenic <sup>1</sup> phthalate (DCHP)		
Demain	11005702	Matria	Dating	Commonte
Domain	Metric 13:	Test Organism Characteristics	High	The Daphnia magna 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 Daphnia 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 Daphnia magna per test concentration container. There were ten contain- ers per test concentration group.
Domain 5: Outcome As	ssessment			
	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 Daphnia were fed Cholrella vulgaris at 0.2 mg of organic content per organism per day. During the exposure, Daphnia were fed Cholrella vulgaris 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 Daphnia 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: Confoundin	g / Variable Co	ntrol		
	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 attri- tion or health outcomes unrelated to exposure. However, there was 20% mortality in the negative control group.
Domain 7: Data Presen	tation and Anal	ysis		
	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.
		Con	tinued on nex	t page

Dicyclohexyl Phthalate

### Environmental Hazard Evaluation

HERO ID: 11803962 Table: 2 of 2

		contir	ued from p	previous page		
Study Citation: Duration:	NITE, (2000 Overall Dura	NITE, (2000). Dicyclohexyl phthalate: Reproduction inhibition test for Daphnia magna (translation). Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days				
Exposure Route,	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)					
Media, Path:						
Taxa, Species, Age:	Invertebrate; Arthropods; Daphnia magna; Juvenile					
Health Outcome:	Reproductive/Teratogenic					
Chemical:	Dicyclohexy	Dicyclohexyl phthalate (DCHP)				
HERO ID:	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 Daphnia magna duri over half of the test, which raises some concern.				a during a 21-d reproduction test. There was 20% mortality in the negative control for		
<b>Overall Qualit</b>	<b>Overall Quality Determination</b>		High			

### Dicyclohexyl Phthalate

#### PUBLIC RELEASE DRAFT December 2024 Environmental Hazard Evaluation

Study Citation:	Sung, H. H.	. Kao, W. Y., Su, Y. J. (2003). Effects and	toxicity of phtha	alate esters to hemocytes of giant freshwater prawn. Macrobrachium rosenbergii.			
J	Aquatic Tox	uatic Toxicology 64(1):25-37. erall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) ustic (frachwater); Cell Culture Media: Net determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Duration:	Overall Dur						
Exposure Route,	Aquatic (fre	shwater); Cell Culture Media; Not determi	ned by study au	thors (i.e., chemical of interest in exposure water, but unable to determine exact			
Media, Path:	uptake route	2)					
Taxa, Species, Age:	Invertebrate	; Arthropods; Macrobrachium rosenbergii; I	Not Applicable (	e.g., fungi or algae studies) or Not Reported			
Health Outcome:	Mechanistic	chanistic-Cell signaling/function					
Chemical:	Dicyclohexy	l phthalate (DCHP)					
HERO ID:	789598	789598					
Domain	Metric Rating Comments						
Domain 1: Test Substan	ce						
	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	DCHP was purchased from Fluka Chem. Co.			
	Metric 3:	Test Substance Purity	Low	Purity was not reported.			
Domain 2: Test Design	Matria 4.	Nagativa Controla	Iliah	Non-time controllo more and			
	Metric 4.	Negative Control Pesponso	High	Regative controls were used.			
	Metric 5:	Randomized Allocation	Low	Organisms were purchased from local prown farms on separate days. The hemocytes			
	Wette 0.	Kandoniized Anocation	LOw	isolated from 5 or 10 prawns were used for the assays, but the authors did not specifi-			
				cally mention random allocation of the hemocytes from different prawns for the various			
				assays.			
Domain 3. Exposure Ch	aracterization						
Domain 5. Exposure en	Metric 7.	Experimental System/Test Media	Medium	The authors reported that the phthalate stocks were separately discolved in acetone and			
	Metric 7.	Preparation	Wiedium	diluted with M-199 (hemocyte-culture medium) to a concentration of 1000 mg/ml. They			
		Topulation		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	High	The exposure was consistent.			
	Metric 0.	Administration Measurement of Test Substance	Low	Exposure concentrations were not reported/measured			
	Wieure 9.	Concentration	LOW	Exposure concentrations were not reported/measured.			
	Metric 10:	Exposure Duration and Frequency	High	An appropriate exposure time was used.			
	Metric 11:	Number of Exposure Groups/	Medium	In Table 1, the authors reported treatment with different concentrations (25, 50 and 100			
		Spacing of Exposure Levels		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			
	Matria 12	Tasting at or Palow Saluhility I init	Madium	assays or only some of the assays.			
	Metric 12:	resung at or Below Solubility Limit	Mealum	I he less substance was below the solubility limit as reported. But the authors did not report the concentration of acetone used to discolve the phthalate and did not measure			
				the phthalate concentration.			

Domain 4: Test Organism

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Dicyclohexyl Phthalate

#### ... continued from previous page **Study Citation:** Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37. **Duration:** Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact **Exposure Route**, Media, Path: uptake route) Taxa, Species, Age: Invertebrate; Arthropods; Macrobrachium rosenbergii; Not Applicable (e.g., fungi or algae studies) or Not Reported **Health Outcome:** Mechanistic-Cell signaling/function Chemical: Dicyclohexyl phthalate (DCHP) **HERO ID:** 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 main- tained 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 pseu- dopodia 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 Co	ontrol		
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 Ana	lysis		
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.
	Cont	inued on next pa	nge

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Dicyclohexyl Phthalate

HERO ID: 789598 Table: 1 of 2

		continu	ed from previ	ous page		
Study Citation:	Sung, H. H. Aquatic Tox	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.				
Duration:	Overall Dura	ation: 0 - 4 days (0-96h); Exposure Duration:	0 - 4 days (0-9	6h)		
Exposure Route.	Aquatic (freshwater): Cell Culture Media: Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Media, Path:	uptake route)					
Taxa, Species, Age:	Invertebrate; Arthropods; Macrobrachium rosenbergii; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-Cell signaling/function					
Chemical:	Dicyclohexyl phthalate (DCHP)					
HERO ID:	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 super-oxide 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

Dicyclohexyl Phthalate

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

Study Citation:	Sung, H. H. Aquatic Tox	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.					
Duration:	Overall Dur	ation: 0 - 4 days (0-96h); Exposure Dura	ation: 0 - 4 days (0-9	6h)			
Exposure Route,	Aquatic (fre	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact					
Media, Path:	uptake route)						
Taxa, Species, Age:	Invertebrate; Arthropods; Macrobrachium rosenbergii; Not Applicable (e.g., fungi or algae studies) or Not Reported						
Health Outcome:	Mechanistic-Cell signaling/function						
Chemical:	Dicyclohexyl phthalate (DCHP)						
HERO ID:	789598						
Domain		Metric	Rating	Comments			
Domain 1: Test Substand	ce						
	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							
Domain 2: Test Design	Metric 4:	Negative Controls	High	Negative controls were used.			
Domain 2: Test Design	Metric 4: Metric 5:	Negative Controls Negative Control Response	High High	Negative controls were used. Biological responses of controls were appropriate.			

assays.

			assaysi	
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	High	Exposure administration was consistent across groups.	
Metric 9:	Administration Measurement of Test Substance	Low	Exposure concentrations were not reported/measured.	
Metric 10:	Concentration 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.	
Continued on next page				

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Dicyclohexyl Phthalate

		conti	nued from previ	ous page	
Study Citation: Duration: Exposure Route, Media, Path: Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	<ul> <li>Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.</li> <li>Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)</li> <li>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)</li> <li>Invertebrate; Arthropods; <i>Macrobrachium rosenbergii</i>; Not Applicable (e.g., fungi or algae studies) or Not Reported</li> <li>Mechanistic-Cell signaling/function</li> <li>Dicyclohexyl phthalate (DCHP)</li> <li>789598</li> </ul>				
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 A	ssessment				
	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 elec- trophoresis, transmission electron microscopy] were reported and appropriate for the outcomes of interest in hemocytes [cell death by necrosis and apoptosis and cell mor- phology 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: Confoundin	g / Variable Co	ntrol			
2 children of Comoundant	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 Presen	tation and Anal	vsis			

Metric 22:	Reporting of Data
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Metric 21:

Metric 23:

Statistical Methods

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.
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.

Statistical analysis was reported but not explained in detail.

Continued on next page ...

Low

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Environmental Hazard Evaluation

HERO ID: 789598 Table: 2 of 2

continued from previous page						
Study Citation:	Sung, H. H., Kao, W. Y., Su, Y. J. (2003). Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn, Macrobrachium rosenbergii. Aquatic Toxicology 64(1):25-37.					
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposu	ure Duration: 0 - 4 days (0-96h)				
Exposure Route,	Aquatic (freshwater); Cell Culture Media; N	Not determined by study authors (i.e., che	mical of interest in exposure water, but unable to determine exact			
Media, Path:	uptake route)					
Taxa, Species, Age:	Invertebrate; Arthropods; Macrobrachium rosenbergii; Not Applicable (e.g., fungi or algae studies) or Not Reported					
Health Outcome:	Mechanistic-Cell signaling/function	Mechanistic-Cell signaling/function				
Chemical:	Dicyclohexyl phthalate (DCHP)					
HERO ID:	789598					
Domain	Metric	Rating	Comments			
Additional Comments:	This study was an in vitro experiment where rosenbergii ) were exposed to BBP, DBP, DE viability assays: (1) Nonspecific cell-mediate (a measure of the initial procedures of either functions), and nitroblue tetrazolium solution toxicity/viability assays included detection o microscopy. This form was used to evaluate	e hemocytes (immune cells) isolated from t EHP, or DCHP. Endpoints encompassed non- ed immune defense response assays include phagocytosis or encapsulation), phenoloxi n (NBT) assay to determine superoxide pro- f cell death via necrosis, detection of cell d hemocyte toxicity/viability due to DCHP.	he hemolymph of 5 to 10 giant freshwater prawn (Macrobrachium specific cell-mediated immune function assays as well as hemocyte d determination of hemocytic adhesion and pseudopodia formation dase activity assay (a measure of pathogen recognition and defense oduction (a measure of highly microbicidal activity); (2) Hemocyte eath via apoptosis, and impacts on cellular morphology assessed by			

**Overall Quality Determination** 

Medium

Dicyclohexyl Phthalate

Study Citation: Duration: Exposure Route, Media Path:	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae (Selenastrum capricornutum) (translation). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)			
Taxa. Species. Age:	Vegetation: 1	Non-vascular Plants: Selenastrum capricorn	<i>utum</i> : Not Appli	cable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Developmen	t/Growth		······································
Chemical:	Dicyclohexy	l phthalate (DCHP)		
HERO ID:	11803966			
Domain		Metric	Rating	Comments
Domain 1: Test Substance	ce			
	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 analyti- cally 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 Cha	aracterization			
	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/	N/A	There was only one exposure concentration in this test (2.00mg/L DCHP).
	Metric 12:	Spacing of Exposure Levels 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 Organish	n 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.
		Contin	nued on next pa	ge

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Dicyclohexyl Phthalate

Name Har.         Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported           Bras. Species, Age:         Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported           Development/Growth         Divelopment/Growth         Comments           Domain         Metric 14:         Acclimatization and Pretreatment Conditions         Little information was provided in the ECHA on the test conditions other than test tem- perature and pH. The culture conditions were not reported.           Metric 13:         Number of Organisms and Replicates per Group         Medium         The initial alga cell concentration appeared to 16 10000 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 17:         Outcome Assessment Methodology         High           Metric 17:         Outcome Assessment Methodology         High         The enduce massessment methodology and creator properted the intended outcome of interest-developmend/growth in terms of growth inhibition of algael cells for 72h.           Metric 18:         Consistency of Outcome Assessment         Low         The study did not provide mach information regarding the outcome assessment process. It is possible the papanee PDF has now information regarding this, but an En- glish translation would be needed to confirm this.           Domain 6:         Confoun	Study Citation: Duration: Exposure Route, Media Path:	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae (Selenastrum capricornutum) (translation). Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h) Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)				
DomainMetricRatingCommentsMetric 14:Acclimatization and Pretreatment ConditionsLowLittle information was provided in the ECHA on the test conditions other than test tem- perature and pH. The culture conditions 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 repitates for the test concentration and six replicates for the controls.Domain 5: Outcome Assessment Metric 17:Adequacy of Test ConditionsLowLittle information on the test conditions and culturing conditions was provided in the Japanese PDF. It was reported in Table 2 of the Japanese PDF. This metric rating could change should a English version become available.Domain 5: Outcome Assessment Metric 17:Outcome Assessment MethodologyHighThe cource assessment methodology address dor reported the intended outcome of interest-development/growth in terms of growth inhibition of algal cells for 72h.Details regarding the execution of the study protocol for outcome assessment methodology address dor reported the intended outcome of interest-development/growth in terms of growth infibrition of algal cells for 72h.Domain 6: Confounding / Variable Control Metric 19:Confounding Variables in Test Design and ProceduresLowThe study did not provide much information regarding the suctomest address of reported. methodology success and groups.Domain 7: Data Presentation and Analysis Metric 21:Statistical MethodsHigh Statistical methods were reported in the Japanese PDF under Table 4. Yukes Statilght #3 software was used to conduct statistical analyses.Domain 7: Data Presentation and Analysis Metric 22:Reporting of DataHigh PD	Taxa, Species, Age: Health Outcome: Chemical: HERO ID:	Vegetation; Non-vascular Plants; <i>Selenastrum capricornutum</i> ; Not Applicable (e.g., fungi or algae studies) or Not Reported Development/Growth Dicyclohexyl phthalate (DCHP) 11803966				
Metric 14;       Acclimatization and Pretreatment Conditions       Low       Little information was provided in the ECHA on the test conditions other than test tem- perature and pH. The culture conditions were not reported.         Metric 15:       Number of Organisms and Replicates per Group       Medium       The initial agal 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 cortols.         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 emperature and pH. The culture conditions and culturing conditions was provided in the ECHA. Only test emperature 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 agal cells for 72h.         Details regarding the execution of the study protocol for outcome assessment process. It is possible the Japanese PDF has more information regarding the study groups.         Domain 6: Confounding / Variable Control Metric 19:       Confounding Variables in Test Design and Procedures Metric 20:       Low       The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.         Domain 7: D	Domain		Metric	Rating	Comments	
Metric 15:       Number of Organisms and Replicates per Group       Medium       The initial agal 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 agla cells for 72h.         Metric 18:       Consistency of Outcome Assessment       Low       Details regarding the execution of the study protocol for outcome assessment were lim- ited. The ECHA did not provide much information regarding the outcome assessment process. It is possible the Japanese PDF has more information regarding the outcome assessment possing and Procedures         Domain 6:       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:       Outcome Surgers and Analysis Metric 21:       Statistical Methods       High       Statistical methods were reported in the study to suggest differences among groups in animal attritio		Metric 14:	Acclimatization and Pretreatment Conditions	Low	Little information was provided in the ECHA on the test conditions other than test tem- perature and pH. The culture conditions were not reported.	
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       Low       Details regarding the excettion 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 the sector of the study protocol for outcome assessment methodology and the confirm this.         Domain 6:       Confounding / Variable Control       Ketric 20:       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.         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.         Domain 7:       Data Presentation and Analysis       High       Statistical methods were reported in talaparese PDF under Table 4. Yukes Statlight #3 software		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.	
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Domain 6: Confounding / Variable Control       Metric 19:       Confounding Variables in Test       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.		Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were lim- ited. 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 En- glish translation would be needed to confirm this.	
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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.		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.	
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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.		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 Selenastrum capricornutum. 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

Dicyclohexyl Phthalate

### Environmental Hazard Evaluation

HERO ID: 11803966 Table: 1 of 1

	•	continued from previous page			
Study Citation:	NITE, (2000). Growth inhibition test of dicyclohexyl phthalate on algae (Selenastrum capricornutum) (translation).				
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure 1	Duration: 0 - 4 days (0-96h)			
Exposure Route,	Aquatic (freshwater); Water; Not determined by	study authors (i.e., chemical of interest	st in exposure water, but unable to determine exact uptake route)		
Media, Path:					
Taxa, Species, Age:	Vegetation; Non-vascular Plants; Selenastrum capricornutum; Not Applicable (e.g., fungi or algae studies) or Not Reported				
Health Outcome:	Development/Growth				
Chemical:	Dicyclohexyl phthalate (DCHP)				
HERO ID:	11803966				
Domain	Metric	Rating	Comments		