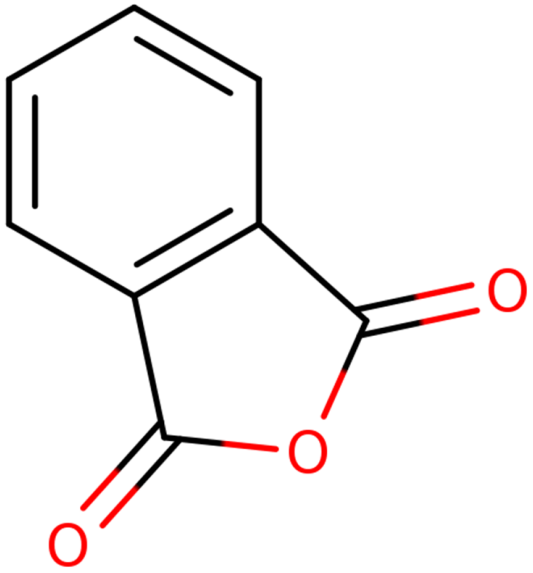
United States
Environmental Protection Agency

March 2026
Office of Chemical Safety and
Pollution Prevention

Data Quality Evaluation Information for Environmental Hazard for Phthalic Anhydride

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 85-44-9



March 2026

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 Phthalic Anhydride*. 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'). Any updated steps in the systematic review process since the publication of the 2021 Draft Systematic Review Protocol are described in the *Draft Systematic Review Protocol for Phthalic Anhydride*.

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.5.10 of the 2021 Draft Systematic Review Protocol and the updated PECO statement in Section 4.6 of the *Draft Systematic Review Protocol for Phthalic Anhydride*. 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 for the target chemicals, namely phthalic anhydride and phthalic acid, are organized by first the relevant habitat (*i.e.*, aquatic vs. terrestrial), then taxa categories (*e.g.*, vertebrates, invertebrates, vegetation) followed by taxonomic groups (*e.g.*, fish, amphibian, mammalian, avian, worms, vascular plants), individual species, and finally exposure duration and health outcome (*e.g.*, mortality) categories relevant to the endpoint being evaluated.

Table of Contents

HERO ID	Reference	Page
Phthalic anhydride		
Habitat: Aquatic (freshwater)		
Taxa: Vertebrates		
Brachydanio rerio		
5353166	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.	**5**
Salmo gairdneri		
5353166	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.	**11**
Habitat: Aquatic (marine)		
Taxa: Plants (Non-vascular)		
Gymnodinium breve		
790296	Wilson, W. B., Giam, C. S., Goodwin, T. E., Aldrich, A., Carpenter, V., Hrung, Y. C. (1978). The toxicity of phthalates to the marine dinoflagellate Gymnodinium breve. Bulletin of Environmental Contamination and Toxicology 20(2):149-154.	**17**
Habitat: Terrestrial		
Taxa: Vertebrates		
Gallus gallus		
94541	Korhonen, A., Hemminki, K., Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. Drug and Chemical Toxicology 6(2):191-207.	**19**
Phthalic acid		
Habitat: Aquatic (freshwater)		
Taxa: Vertebrates		
Oncorhynchus mykiss		

11138764 [Redacted] (1996). Phthalic acid: Acute toxicity test with rainbow trout (Oncorhynchus mykiss) under static conditions: Lab project number: 1026.013.103. **23**

Taxa: Invertebrates

Chironomus plumosus

1332972 Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus). **27**

Daphnia magna

789536 Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043. **31**

Taxa: Plants (Non-vascular)

Desmodesmus subspicatus

5353164 Services,, B.I. (2004). Internal report: Alga, growth inhibition test of phthalic acid. **34**

Pseudokirchneriella subcapitata

789536 Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043. **37**

Habitat: Terrestrial

Taxa: Plants (Vascular)

Lilium davidii var. unicolor

6824698 Hua, C. P., Xie, Z. K., Wu, Z. J., Zhang, Y. B., Guo, Z. H., Qiu, Y., Wang, L., Wang, Y. J. (2019). The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (Lilium davidii var. unicolor). HortScience 54(2):253-261. **40**

Malus prunifolia (crabapple)

6813707 Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494. **44**

Nicotiana tabacum

6968271 Huiyong, Y., Hongbo, L., Guoming, S., Sampietro, D. A., Xinxin, G. (2014). Effects of allelochemicals from tobacco root exudates on seed germination and seedling growth of tobacco. Allelopathy Journal 33(1):107-119. **54**

Taxa: Other

Sclerotinia sclerotiorum

6826077 Loffredo, E., Traversa, A. (2014). Soil and compost humic fractions regulate the response of Sclerotinia sclerotiorum to exogenously added allelochemical compounds. Biology and Fertility of Soils 50(8):1281-1290. **58**

Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The phthalic anhydride was identified by name only.
	Metric 2:	Test Substance Source	Low	It was reported that the test substance was supplied by Merck, but it was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be >- 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. In the case of chemicals that needed aid in dissolution, such as phthalic anhydride, study authors reported the use of a solvent (DMSO).
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported. Only hazard values were reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the eggs were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test solution was not described in detail. The test system was reported to be semi-static with test solutions renewed three times a week. Stock solutions were reported to be prepared fresh at each renewal period, but that preparation method was not reported. Little was reported on preparations to minimize loss of test substance, and test concentrations were not measured at any point in the study.
	Metric 8:	Consistency of Exposure Administration	Low	More details are needed regarding the exposure administration details to make a judgement on this metric. The tests were carried out in 60mL glass test vessels with 50mL of test solution. Since the test substance preparation was not reported, and the test concentrations did not appear to be measured, it is unclear if the test concentrations remained close to nominal concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations were not reported to be measured at any point in the study.

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Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	High	The early life stage study with zebrafish embryos was reported to be seven days in duration. This appeared appropriate for the outcomes of interest - comparing/contrasting the difference in sensitivity. The study authors state, "The difference in sensitivity between the 60-day ELS test on rainbow trout and the 7-day ELS lest on zebra fish is relatively small. A similar conclusion was drawn in a comparative toxicological study on fish by Fogels and Sprague (1977). Therefore it can be concluded that the short-term ELS test on zebra fish is a suitable short-cut method both for environmental hazard assessment and for screening of direct-acting human teratogens. It deserves further optimization and standardization within the framework of ISO, OECD, and EEC. A first step has been taken in a Danish-Dutch proposal (Kristensen et al., 1988)."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors reported the use of 5-7 toxicant concentrations. The range between concentrations was reported to be 3.2. However, the test concentrations were not actually reported, so it is unclear how many were used for each test chemical and whether the highest and lowest test concentrations were appropriate for the study.
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentrations were not actually reported, so it is unclear if any of the test concentrations exceeded the water solubility limit. The study authors did report the use of DMSO to aid in dissolution, but solvent control results were not reported.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The zebrafish eggs were obtained from cultures in the performing laboratory. The embryos were in the blastula stage (2-4h after spawning).
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Culture conditions were not reported in detail, so it is unclear if pretreatment conditions were similar to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 60 embryos per test concentration, but there were not replicates for this portion of the study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	For the duration of the study, embryos were kept at 25C with a photoperiod of 12L:12D. No food was provided to the larvae. The reconstituted water characteristics were reported. pH and oxygen levels were monitored.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology addressed or reported the intended outcome(s) of interest–mortality in the form of an LC50 value. However, the LC50 value was 560 mg/L (with 95%CIs of 320-1000) while the LOAEC concentration was 1000 mg/L. Although the statistical methodology was reported, the LC50 extrapolation is questionable.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome was assessed consistently across study groups. Morality was assessed daily, and any dead larvae were counted and removed daily.
Domain 6: Confounding / Variable Control				

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Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	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. Pre-treatment conditions for the zebrafish culture were not reported in detail.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported and described adequately in the ”Calculation and Statistics” section. LC50 and 95% confidence intervals were calculated according to Litchfield and Wilcoxon or Kooyman. Concentrations were tested against the blank control by means of a x’ test (Sokal and Rohlf, 1981). Differences in mean length and weight between treatments and control were tested using procedures described by Williams (197 1, 1972).
	Metric 22:	Reporting of Data	Low	Data were not presented for each exposure level and control response. Only LC50 values and their confidence intervals and LOECs were reported, although the reported LC50 was less than the LOAEC.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study authors did not report any unexpected outcomes. Confidence intervals were reported. It was unclear how the LC50 was less than the reported LOAEC.

Additional Comments: This evaluation is for the 7 day ELS zebrafish study in which zebrafish embryos were exposed to 5-7 concentrations of phthalic anhydride. Mortality was one of the outcomes of interest, and a 7d LC50 value was determined with confidence intervals. This was reported in Table 2.

Overall Quality Determination — Medium

Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The phthalic anhydride was identified by name only.
	Metric 2:	Test Substance Source	Low	It was reported that the test substance was supplied by Merck, but it was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be >- 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. In the case of chemicals that needed aid in dissolution, such as phthalic anhydride, study authors reported the use of a solvent (DMSO).
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported. Only hazard values were reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the eggs were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test solution was not described in detail. The test system was reported to be semi-static with test solutions renewed three times a week. Stock solutions were reported to be prepared fresh at each renewal period, but that preparation method was not reported. Little was reported on preparations to minimize loss of test substance, and test concentrations were not measured at any point in the study.
	Metric 8:	Consistency of Exposure Administration	Low	More details are needed regarding the exposure administration details to make a judgement on this metric. The tests were carried out in 60mL glass test vessels with 50mL of test solution. Since the test substance preparation was not reported, and the test concentrations did not appear to be measured, it is unclear if the test concentrations remained close to nominal concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations were not reported to be measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The early life stage study with zebrafish embryos was reported to be seven days in duration. This appeared appropriate for the outcomes of interest. The early life stage study with zebrafish embryos was reported to be seven days in duration. This appeared appropriate for the outcomes of interest - comparing/contrasting the difference in sensitivity. The study authors state, "The difference in sensitivity between the 60-day ELS test on rainbow trout and the 7-day ELS lest on zebra fish is relatively small. A similar conclusion was drawn in a comparative toxicological study on fish by Fogels and Sprague (1977). Therefore it can be concluded that the short-term ELS test on zebra fish is a suitable short-cut method both for environmental hazard assessment and for screening of direct-acting human teratogens. It deserves further optimization and standardization within the framework of ISO, OECD, and EEC. A first step has been taken in a Danish-Dutch proposal (Kristensen et al., 1988)."

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Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors reported the use of 5-7 toxicant concentrations. The range between concentrations was reported to be 3.2. However, the test concentrations were not actually reported, so it is unclear how many were used for each test chemical and whether the highest and lowest test concentrations were appropriate for the study.
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentrations were not actually reported, so it is unclear if any of the test concentrations exceeded the water solubility limit. The study authors did report the use of DMSO to aid in dissolution, but solvent control results were not reported.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The zebrafish eggs were obtained from cultures in the performing laboratory. The embryos were in the blastula stage (2-4h after spawning).
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Culture conditions were not reported in detail, so it is unclear if pretreatment conditions were similar to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 60 embryos per test concentration, but there were not replicates for this portion of the study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	For the duration of the study, embryos were kept at 25C with a photoperiod of 12L:12D. No food was provided to the larvae. The reconstituted water characteristics were reported. pH and oxygen levels were monitored.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed or reported the intended outcomes of interest–total embryotoxicity was reported in Table 2. This is reported to be a combined outcome for lethality and malformations.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the outcome assessment were limited. It was reported that surviving fish at the end of the study were assessed macroscopically for malformations under binocular amplification. How this assessment was conducted was unclear.
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. Pre-treatment conditions for the zebrafish culture were not reported in detail.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				

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Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Brachydanio rerio*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	Statistical methods were reported and described adequately in the ”Calculation and Statistics” section. EC50 and 95% confidence intervals were calculated according to Litchfield and Wilcoxon or Kooyman.
	Metric 22:	Reporting of Data	Low	Data were not presented for each exposure level and control response. Only EC50 values and their confidence intervals and LOECs were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

Additional Comments: This evaluation is for the 7 day ELS zebrafish study in which zebrafish embryos were exposed to 5-7 concentrations of phthalic anhydride. Developmental outcomes were assessed and reported partially as ”total embryotoxicity.” However, this outcome was a combination of mortality and malformation outcomes. The actual malformations assessed were not reported.

Overall Quality Determination Medium

Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The phthalic anhydride was identified by name only.
	Metric 2:	Test Substance Source	Low	It was reported that the test substance was supplied by Merck, but it was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be >- 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. In the case of chemicals that needed aid in dissolution, such as phthalic anhydride, study authors reported the use of a solvent (DMSO).
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported. Only hazard values were reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the eggs were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test solution was not described in detail. The test system was reported to be semi-static with test solutions renewed three times a week. Stock solutions were reported to be prepared fresh at each renewal period, but that preparation method was not reported. Little was reported on preparations to minimize loss of test substance, and test concentrations were not measured at any point in the study.
	Metric 8:	Consistency of Exposure Administration	Low	More details are needed regarding the exposure administration details to make a judgement on this metric. The tests were carried out in 15L glass aquaria with 10L of test solution. Since the test substance preparation was not reported, and the test concentrations did not appear to be measured, it is unclear if the test concentrations remained close to nominal concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations were not reported to be measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The early life stage study with rainbow trout was reported to be 60d. This appeared adequate for the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Study authors reported the use of 5-7 toxicant concentrations. The range between concentrations was reported to be 3.2. However, the test concentrations were not actually reported, so it is unclear how many were used for each test chemical and whether the highest and lowest test concentrations were appropriate for the study.

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Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentrations were not actually reported, so it is unclear if any of the test concentrations exceeded the water solubility limit. The study authors did report the use of DMSO to aid in dissolution, but solvent control results were not reported.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Fertilized rainbow trout eggs were obtained from a fish hatchery in Vaassen in Gelderland, Netherlands. The eggs were used for test initiation about 3h after fertilization.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study authors did not report if acclimation occurred at any point during the rainbow trout study.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 100 eggs per test chamber. It was reported that there were two replicates per test concentration, but this is lower than is typical. OECD Test Guideline 210 for fish early life stage toxicity tests, it is recommended to use at least four replicates per treatment group, including the control group. Note - guidelines should not be interpreted as absolute
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	For the duration of the study, embryos were kept at 10C. During embryogenesis, the eggs were kept in the dark, but once the hatched, a photoperiod of 12L:12D was maintained. Water characteristics were reported, but no information regarding a feeding regimen was reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome(s) of interest–mortality in the form of an LC50 value. 95% confidence intervals were reported.
	Metric 18:	Consistency of Outcome Assessment	Medium	It was reported the aquaria were regularly inspected for dead larvae, but it was not reported how often this occurred.
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. Study authors did not report if the test organisms were acclimated at any point in the study.
	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 and described adequately in the ”Calculation and Statistics” section. LC50 and 95% confidence intervals were calculated according to Litchfield and Wilcoxon or Kooyman.

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Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	Low	Data were not presented for each exposure level and control response. Only LC50 values and their confidence intervals and LOECs were reported (Table 3).
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Confidence intervals were reported.

Additional Comments: This evaluation is for the 60 day ELS rainbow trout study in which rainbow trout embryos were exposed to concentrations of phthalic anhydride. Mortality was one of the outcomes of interest, and a 60d LC50 value was determined with confidence intervals. This was reported in Table 3.

Overall Quality Determination Medium

Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Development/Growth
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HERO ID:	5353166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The phthalic anhydride was identified by name only.
	Metric 2:	Test Substance Source	Low	It was reported that the test substance was supplied by Merck, but it was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be >- 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control. In the case of chemicals that needed aid in dissolution, such as phthalic anhydride, study authors reported the use of a solvent (DMSO).
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported. Only hazard values were reported.
	Metric 6:	Randomized Allocation	Low	It was not reported how the eggs were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test solution was not described in detail. The test system was reported to be semi-static with test solutions renewed three times a week. Stock solutions were reported to be prepared fresh at each renewal period, but that preparation method was not reported. Little was reported on preparations to minimize loss of test substance, and test concentrations were not measured at any point in the study.
	Metric 8:	Consistency of Exposure Administration	Low	More details are needed regarding the exposure administration details to make a judgement on this metric. The tests were carried out in 15L glass aquaria with 10L of test solution. Since the test substance preparation was not reported, and the test concentrations did not appear to be measured, it is unclear if the test concentrations remained close to nominal concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations were not reported to be measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The early life stage study with rainbow trout was reported to be 60d. This appeared adequate for the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range between concentrations was reported to be 3.2. However, the test concentrations were not actually reported, so it is unclear how many were used for each test chemical and whether the highest and lowest test concentrations were appropriate for the study.
	Metric 12:	Testing at or Below Solubility Limit	Low	The exposure concentrations were not actually reported, so it is unclear if any of the test concentrations exceeded the water solubility limit. The study authors did report the use of DMSO to aid in dissolution, but solvent control results were not reported.
Domain 4: Test Organism				

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Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Fertilized rainbow trout eggs were obtained from a fish hatchery in Vaassen in Gelderland, Netherlands. The eggs were used for test initiation about 3h after fertilization.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Study authors did not report if acclimation occurred at any point during the rainbow trout study.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were 100 eggs per test chamber. It was reported that there were two replicates per test concentration, but this is lower than is typical. OECD Test Guideline 210 for fish early life stage toxicity tests, it is recommended to use at least four replicates per treatment group, including the control group. Note - guidelines should not be interpreted as absolute
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	For the duration of the study, embryos were kept at 10C. During embryogenesis, the eggs were kept in the dark, but once the hatched, a photoperiod of 12L:12D was maintained. Water characteristics were reported, but no information regarding a feeding regimen was reported.
	Metric 17:	Outcome Assessment Methodology	Medium	The outcome assessment methodology partially addressed or reported the intended outcomes of interest–length and weight were assessed at the end of the study, and embryotoxicity was reported. Embryotoxicity was reported to include mortality and malformation data. There was no singular data on malformations only.
	Metric 18:	Consistency of Outcome Assessment	Medium	Fish were assessed at the end of the 60 day test period for malformations, length, and weight. The procedure for assessing malformations was not reported in detail.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. Study authors did not report if the test organisms were acclimated at any point in the study.
	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 and described adequately in the ”Calculation and Statistics” section. LC50 and 95% confidence intervals were calculated according to Litchfield and Wilcoxon or Kooyman.
	Metric 22:	Reporting of Data	Low	Data were not presented for each exposure level and control response. Only EC50 values and their confidence intervals and LOECs were reported (Table 3).
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

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Study Citation:	Leeuwen, Van, C. J., Grootelaar, E. M., Niebeek, G. (1990). Fish embryos as teratogenicity screens: A comparison of embryotoxicity between fish and birds. Ecotoxicology and Environmental Safety 20(1):42-52.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Salmo gairdneri*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	5353166

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the 60 day ELS rainbow trout study in which rainbow trout embryos were exposed to concentrations of phthalic anhydride. Development/growth was one of the outcomes of interest, and a 60d LEC50 value was determined with confidence intervals along with LOEC values. LOECs for length, weight, and embryotoxicity were reported. This was reported in Table 3.		

Overall Quality Determination Medium

Study Citation:	Wilson, W. B., Giam, C. S., Goodwin, T. E., Aldrich, A., Carpenter, V., Hrung, Y. C. (1978). The toxicity of phthalates to the marine dinoflagellate Gymnodinium breve. Bulletin of Environmental Contamination and Toxicology 20(2):149-154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Gymnodinium breve*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	790296

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	Substance identified as disodium salt of phthalic acid; no source or CASRN reported.
	Metric 2:	Test Substance Source	Low	Source of test substance was not reported.
	Metric 3:	Test Substance Purity	Low	Purity of test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The use of controls was described in the methods.
	Metric 5:	Negative Control Response	Uninformative	No response for controls was reported throughout the study.
	Metric 6:	Randomized Allocation	Low	The authors did not report random allocation of organisms, cultures were grown in the lab.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Limited details on the study design were reported.
	Metric 8:	Consistency of Exposure Administration	Medium	Preparation of phthalates for exposures was described in detail in the methods.
	Metric 9:	Measurement of Test Substance Concentration	Uninformative	Concentrations were not analytically measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure frequency was appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Each exposure concentration was completed in triplicate, with sufficient spacing between concentrations.
	Metric 12:	Testing at or Below Solubility Limit	High	Saturated solutions were used and diluted to applicable test concentrations.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were cultured in the lab, source is cited (Gates and Wilson 1960).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Cultures were transferred 14 days prior to experiment for acclimatization.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Cultures contained 1000-5000/mL.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Limited details about the experimental conditions were provided.
	Metric 17:	Outcome Assessment Methodology	Low	Tables did not include phthalic acid results, a short statement in the results was reported for outcomes.

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Study Citation:	Wilson, W. B., Giam, C. S., Goodwin, T. E., Aldrich, A., Carpenter, V., Hrung, Y. C. (1978). The toxicity of phthalates to the marine dinoflagellate Gymnodinium breve. Bulletin of Environmental Contamination and Toxicology 20(2):149-154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (marine); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Gymnodinium breve*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	790296

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Consistency among groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Authors did not report sufficient information to allow comparison.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistics were not reported.
	Metric 22:	Reporting of Data	Low	Limited details reported on the outcome of phthalic acid in the study.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not reported.

Additional Comments: *Please note: The actual test chemical tested in the reference was phthalic acid, disodium salt.

Overall Quality Determination — Low

Study Citation:	Korhonen, A., Hemminki, K., Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. Drug and Chemical Toxicology 6(2):191-207.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Injection
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus*; White Leghorn; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	94541; Linked HERO ID(s): 94541, 548796

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	Low	The test chemical was obtained from the rubber factory of Oy Nokia Ab, Nokia, Finland. There was no mention of the chemical being analytically verified.
	Metric 3:	Test Substance Purity	Medium	Authors mentioned the PHTA was of technical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent (acetone) control group was included.
	Metric 5:	Negative Control Response	High	There was <10% mortality in the acetone control group.
	Metric 6:	Randomized Allocation	Low	Authors did not report if the embryos were randomly distributed to the test groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Details were vague on the test system. Embryos were kept in a commercial type of incubator. The method of injection was described in earlier work by the study authors (not included in this paper). Preparation of the test chemical prior to injection was not well described. It was unclear if or when acetone was used for PHTA. If a solvent solution was needed, the exact volume of acetone used was not reported.
	Metric 8:	Consistency of Exposure Administration	Low	Since the experimental system was not described in great detail, it is uncertain whether any inconsistencies may have occurred.
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no report of measuring test concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The incubation was terminated 11 days after the injection and survival was then assessed.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were four dose groups tested. The range was adequate to calculate an LD50. It was not mentioned why those specific doses were chosen prior to test start.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Authors mentioned using acetone as a solvent for test solutions when necessary. It was not specifically stated if acetone was used with PHTA. However, since PHTA is only slightly soluble in water, acetone was likely used.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The white leghorn chicken eggs were obtained from the hatchery of Siipikarjanhoitajain Liitto ry, Hameenlinna, Finland.

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Study Citation:	Korhonen, A., Hemminki, K., Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. Drug and Chemical Toxicology 6(2):191-207.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Injection
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus*; White Leghorn; Embryo
Health Outcome:	Mortality
Chemical:	Phthalic anhydride (PAD)
HERO ID:	94541; Linked HERO ID(s): 94541, 548796

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not mentioned if the eggs were specifically acclimated to test conditions prior to the injection. However, authors reported the incubation conditions, which were likely the same for the three days prior to injections (14 total incubation days).
	Metric 15:	Number of Organisms and Replicates per Group	Low	The highest test dose had 20 eggs and the other three doses each had 30 eggs. There was no mention of replicates per treatment dose.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Some incubation conditions were reported. During the test, the eggs were turned two to four times per day. There was no specific mention of acclimation prior to the chemical injection.
	Metric 17:	Outcome Assessment Methodology	High	Embryo death was assessed throughout the test.
	Metric 18:	Consistency of Outcome Assessment	Medium	Two days after the injection, eggs were examined, dead embryos were counted and then discarded. Then every second or third day until the end of the test, the eggs were candled and the dead embryos counted.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There were some details lacking on conditions during the test and it was not mentioned if the eggs were specifically acclimated prior to the chemical injection.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences in eggs between treatment doses that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Hazard effects (LD50s) were derived so it is assumed statistical analyses were run. However, the statistical methods used were not described.
	Metric 22:	Reporting of Data	High	Mortality data was shown in Table 1. Table 2 has the LD50 results.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not provided in the result tables.

Additional Comments: This evaluation is for the assessment of embryo mortality after injection of PHTA.

Overall Quality Determination Medium

Study Citation:	Korhonen, A., Hemminki, K., Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. Drug and Chemical Toxicology 6(2):191-207.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Injection
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus*; White Leghorn; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	94541; Linked HERO ID(s): 94541, 548796

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified by name only.
	Metric 2:	Test Substance Source	Low	The test chemical was obtained from the rubber factory of Oy Nokia Ab, Nokia, Finland. There was no mention of the chemical being analytically verified.
	Metric 3:	Test Substance Purity	Medium	Authors mentioned the PHTA was of technical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent (acetone) control group was included.
	Metric 5:	Negative Control Response	High	There was <10% malformations in the acetone control group.
	Metric 6:	Randomized Allocation	Low	Authors did not report if the embryos were randomly distributed to the test groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Details were vague on the test system. Embryos were kept in a commercial type of incubator. The method of injection was described in earlier work by the study authors (not included in this paper). Preparation of the test chemical prior to injection was not well described. It was unclear if or when acetone was used for PHTA. If a solvent solution was needed, the exact volume of acetone used was not reported.
	Metric 8:	Consistency of Exposure Administration	Low	Since the experimental system was not described in great detail, it is uncertain whether any inconsistencies may have occurred.
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no report of measuring test concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The incubation was terminated 11 days after the injection and external malformations were assessed in the remaining eggs.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were four dose groups tested. The range was adequate to calculate an ED50 and assess embryo malformations. It was not mentioned why those specific doses were chosen prior to test start.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Authors mentioned using acetone as a solvent for test solutions when necessary. It was not specifically stated if acetone was used with PHTA. However, since PHTA is only slightly soluble in water, acetone was likely used.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The white leghorn chicken eggs were obtained from the hatchery of Siipikarjanhoitajain Liitto ry, Hameenlinna, Finland.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not mentioned if the eggs were specifically acclimated to test conditions prior to the injection. However, authors reported the incubation conditions, which were likely the same for the three days prior to injections (14 total incubation days).

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Study Citation:	Korhonen, A., Hemminki, K., Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. Drug and Chemical Toxicology 6(2):191-207.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Injection
Taxa, Species, Age:	Vertebrate; Avian; *Gallus gallus*; White Leghorn; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic anhydride (PAD)
HERO ID:	94541; Linked HERO ID(s): 94541, 548796

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	The highest test dose had 20 eggs and the other three doses each had 30 eggs. There was no mention of replicates per treatment dose.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Some incubation conditions were reported. During the test the eggs were turned two to four times per day. There was no specific mention of acclimation prior to the chemical injection.
	Metric 17:	Outcome Assessment Methodology	High	Embryo malformations were assessed throughout the test.
	Metric 18:	Consistency of Outcome Assessment	Medium	Two days after the injection, eggs were examined, dead embryos were counted and then discarded. Then every second or third day until the end of the test, the eggs were candled and the dead embryos were checked for external malformations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There were some details lacking on conditions during the test and it was not mentioned if the eggs were specifically acclimated prior to the chemical injection.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information reported to suggest differences in eggs between treatment doses that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Hazard effects (ED50 and LD50s) were derived so it is assumed statistical analyses were run. However, the statistical methods used were not described.
	Metric 22:	Reporting of Data	High	Malformation data was shown in Tables 1 and 3. The dose-response curve of malformed embryos is shown in Figure 1. Table 2 has the ED50 results.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not provided in the result tables or the figure.

Additional Comments: This evaluation is for the assessment of embryo malformations after injection of PHTA.

Overall Quality Determination — Medium

Study Citation:	[Redacted] (1996). Phthalic acid: Acute toxicity test with rainbow trout (Oncorhynchus mykiss) under static conditions: Lab project number: 1026.013.103.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Aquatic (freshwater); N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oncorhynchus mykiss*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Other (please specify below) (Outcome 1)
Chemical:	Phthalic acid
HERO ID:	11138764

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	There are no concerns regarding the identity of the test substance.
	Metric 2:	Test Substance Source	High	There are no concerns regarding the test substance source or identity (CBI claimed).
	Metric 3:	Test Substance Purity	High	There are no concerns regarding test substance purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	There are no concerns regarding a negative control.
	Metric 5:	Negative Control Response	High	There are no concerns regarding the negative control response.
	Metric 6:	Randomized Allocation	Medium	There are no concerns regarding random allocation of test organisms to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	There are no concerns regarding experimental system/test media preparation.
	Metric 8:	Consistency of Exposure Administration	High	There are no concerns for consistency of exposure administration.
	Metric 9:	Measurement of Test Substance Concentration	Medium	There are some concerns regarding measurements of the test substance concentration (CBI claimed), but these concerns are unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Duration and Frequency	High	There are no concerns about the study exposure duration and frequency.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There are no concerns for the number or spacing of exposure levels.
	Metric 12:	Testing at or Below Solubility Limit	High	There are no concerns regarding the solubility of the test substance.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	There are no concerns about the characteristics of the test organism.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	There are no acclimatization or pre-treatment concerns.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are no concerns about the number of organisms or replicates per group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There are no concerns about the adequacy of the test conditions.
	Metric 17:	Outcome Assessment Methodology	High	There are no concerns regarding the outcome assessment methodology.

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Study Citation:	[Redacted] (1996). Phthalic acid: Acute toxicity test with rainbow trout (Oncorhynchus mykiss) under static conditions: Lab project number: 1026.013.103.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Aquatic (freshwater); N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oncorhynchus mykiss*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Other (please specify below) (Outcome 1)
Chemical:	Phthalic acid
HERO ID:	11138764

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	There are no concerns about consistency of the outcome assessment.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There are no concerns about potentially confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	High	There are no concerns about outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	This metric is not applicable to this study.
	Metric 22:	Reporting of Data	High	There are no concerns about reporting of data.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexplained outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	[Redacted] (1996). Phthalic acid: Acute toxicity test with rainbow trout (Oncorhynchus mykiss) under static conditions: Lab project number: 1026.013.103.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Aquatic (freshwater); N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oncorhynchus mykiss*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Other (please specify below) (Outcome 2)
Chemical:	Phthalic acid
HERO ID:	11138764

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	There are no concerns regarding the identity of the test substance.
	Metric 2:	Test Substance Source	High	There are no concerns regarding the test substance source or identity (CBI claimed)
	Metric 3:	Test Substance Purity	High	There are no concerns regarding test substance purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	There are no concerns regarding a negative control.
	Metric 5:	Negative Control Response	High	There are no concerns regarding the negative control response.
	Metric 6:	Randomized Allocation	Medium	There are no concerns regarding random allocation of test organisms to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	There are no concerns regarding the experimental system or preparation of the test media.
	Metric 8:	Consistency of Exposure Administration	High	There are no concerns regarding consistency of exposure administration.
	Metric 9:	Measurement of Test Substance Concentration	Medium	There are some concerns regarding measurements of the test substance concentration (CBI claimed), but these concerns are unlikely to have a substantial impact on the study results.
	Metric 10:	Exposure Duration and Frequency	High	There are no concerns about the study exposure duration and frequency.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There are no concerns for the number or spacing of exposure levels.
	Metric 12:	Testing at or Below Solubility Limit	High	There are no concerns regarding the solubility of the test substance.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	There are no concerns about the characteristics of the test organism.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	There are no acclimatization or pre-treatment concerns.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There are no concerns about the number of organisms or replicates per group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There are no concerns about the adequacy of the test conditions.
	Metric 17:	Outcome Assessment Methodology	High	There are no concerns regarding the outcome assessment methodology.
	Metric 18:	Consistency of Outcome Assessment	High	There are no concerns about consistency of the outcome assessment.

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Study Citation:	[Redacted] (1996). Phthalic acid: Acute toxicity test with rainbow trout (Oncorhynchus mykiss) under static conditions: Lab project number: 1026.013.103.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Aquatic (freshwater); N/A (e.g., injection); Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vertebrate; Fish; *Oncorhynchus mykiss*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Other (please specify below) (Outcome 2)
Chemical:	Phthalic acid
HERO ID:	11138764

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There are no concerns about potentially confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	High	There are no concerns about outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	This metric is not applicable to this study.
	Metric 22:	Reporting of Data	High	There are no concerns about reporting of data.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexplained outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); *Chironomus plumosus*; Larvae
Health Outcome:	Mortality
Chemical:	Phthalic acid
HERO ID:	1332972

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	Phthalic acid was obtained from Monsanto Chemical Co.
	Metric 3:	Test Substance Purity	Low	The purity of PA was reported to be > 90%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Controls were reported to have been incorporated in the experimental design but results were not reported.
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported.
	Metric 6:	Randomized Allocation	Low	The study did not report how the organisms were allocated."Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static test 10 late-third and early-fourth instar larvae were exposed to concentrations of phthalate compounds for 48 h in 250 ml of solution in glass jars."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Methods were cited as published in the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)."Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)."
	Metric 8:	Consistency of Exposure Administration	Low	Details on the exposure administration were not reported.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported or analytically verified.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was appropriate for an acute toxicity study, 48 hr.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of organisms (10) per treatment was appropriate, while there was an insufficient number of concentrations tested.
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	PA was dissolved in ethanol and/or acetone to increase solubility. The solvent concentration exceeded the recommended value of 0.5 mg/L for acute studies, as suggested by the Committee on Methods for Toxicity Tests with Aquatic Organisms.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were accurately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	The culturing of test organisms was described in the methods.

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Study Citation:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); *Chironomus plumosus*; Larvae
Health Outcome:	Mortality
Chemical:	Phthalic acid
HERO ID:	1332972

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	"Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests 10 late-third and early-fourth instar larvae were exposed to concentrations of phthalate compounds for 48 h in 250 ml of solution in glass jars."
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Methods used were described in Committee on Methods for Toxicity Tests with Aquatic Organisms.
	Metric 17:	Outcome Assessment Methodology	Low	Phthalic acid was reported to have an EC50 and LC50 >72 mg/L.
	Metric 18:	Consistency of Outcome Assessment	Low	Details were limited. Solubility was an issue throughout and excessive solvent was used.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow for comparison among the phthalic acid exposures.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was reported to suggest differences.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not described adequately. In the acknowledgements section, it was indicated that "Appreciation is extended to Dr. Jean Sebaugh for her help in statistical analysis."
	Metric 22:	Reporting of Data	Low	The outcome for phthalic acid was reported in a table for EC50 and LC50, beyond the table no graphs or details were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study did not include measures of variables for phthalic acid.

Additional Comments: None

Overall Quality Determination — Uninformative

Study Citation:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); *Chironomus plumosus*; Larvae
Health Outcome:	Immobilization
Chemical:	Phthalic acid
HERO ID:	1332972

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Substance was identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	Phthalic acid was obtained from Monsanto Chemical Co.
	Metric 3:	Test Substance Purity	Low	The purity of PA was reported to be > 90%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Controls were reported to have been incorporated in the experimental design but results were not reported.
	Metric 5:	Negative Control Response	Low	Negative control responses were not reported.
	Metric 6:	Randomized Allocation	Low	The study did not report how the organisms were allocated."Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static test 10 late-third and early-fourth instar larvae were exposed to concentrations of phthalate compounds for 48 h in 250 ml of solution in glass jars."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Methods were cited as published in the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)."Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)."
	Metric 8:	Consistency of Exposure Administration	Low	Details on the exposure administration were not reported.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not reported or analytically verified.
	Metric 10:	Exposure Duration and Frequency	High	The test duration was appropriate for an acute toxicity study, 48 hr.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The number of organisms (10) per treatment was appropriate, while there was an insufficient number of concentrations tested.
	Metric 12:	Testing at or Below Solubility Limit	Uninformative	PA was dissolved in ethanol and/or acetone to increase solubility. The solvent concentration exceeded the recommended value of 0.5 mg/L for acute studies, as suggested by the Committee on Methods for Toxicity Tests with Aquatic Organisms.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were accurately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	The culturing of test organisms was described in the methods.

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Study Citation:	Streufort, J. M. (1978). Some effects of two phthalic acid esters on the life cycle of the midge (Chironomus plumosus).
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Other Invertebrate (e.g., sea urchins, ciliates, rotifers); *Chironomus plumosus*; Larvae
Health Outcome:	Immobilization
Chemical:	Phthalic acid
HERO ID:	1332972

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	"Acute toxicity tests were conducted according to procedures recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). In static tests 10 late-third and early-fourth instar larvae were exposed to concentrations of phthalate compounds for 48 h in 250 ml of solution in glass jars."
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Methods used were described in Committee on Methods for Toxicity Tests with Aquatic Organisms.
	Metric 17:	Outcome Assessment Methodology	Low	Phthalic acid was reported to have an EC50 and LC50 >72 mg/L.
	Metric 18:	Consistency of Outcome Assessment	Low	Details were limited. Solubility was an issue throughout and excessive solvent was used.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow for comparison among the phthalic acid exposures.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information was reported to suggest differences.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was not described adequately. In the acknowledgements section, it was indicated that "Appreciation is extended to Dr. Jean Sebaugh for her help in statistical analysis."
	Metric 22:	Reporting of Data	Low	The outcome for phthalic acid was reported in a table for EC50 and LC50, beyond the table no graphs or details were reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study did not include measures of variables for phthalic acid.

Additional Comments: This evaluation form is for the immobilization outcome. This was reported in Table 4 as an EC50 value. Researches assessed both immobilization and mortality.

Overall Quality Determination — Uninformative

Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	CASRN 88-99-3 and purity >99% were identified
	Metric 2:	Test Substance Source	High	the source as identified and verified at the manufacturer or in the study. The diesters were Merck products, purchased from MerckEurolab (Stockholm, Sweden), and the monoesters were synthesized from phthalic acid anhydride and deficient of the alcohol of interest [4]. In short, the main recipe was as follows:Equimolar amounts of phthalic acid anhydride and the alcoholwere refluxed in toluene. After cooling, the mixture was extractedwith acidified water. The organic phase was concentratedin a rotary evaporator, and the products were recrystallized.Ethyl acetate used for extractions during the chemicalanalyses was of chromatography grade. All other chemicalsused were analytical grade, and MilliQt water (Millipore, Bedford,MA, USA) was used throughout the study.
	Metric 3:	Test Substance Purity	High	Purity was reported (table 1) as >99%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	the appropriate control groups were utilized for this bioassay. Each test consisted of five to eight concentrations of test solution and a control group of eight replicates.
	Metric 5:	Negative Control Response	High	Control mortality was <5%. Tests were repeated if the mortality in the control group exceeded 5%.
	Metric 6:	Randomized Allocation	Low	random allocation was not specifically stated
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was reported. Equimolar amounts of phthalic acid anhydride and the alcohol were refluxed in toluene. After cooling, the mixture was extracted with acidified water. The organic phase was concentrated in a rotary evaporator, and the products were recrystallized. Ethyl acetate used for extractions during the chemical analyses was of chromatography grade. The test strategy included range-finding tests covering several decades of concentrations and definitive tests to establish EC values and the corresponding 95% confidence intervals.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent. No inconsistencies reported. The 48-h immobilization tests with crustaceans D. magna were performed in accordance with the International Organization for Standardization (ISO) standard [14] at pH 7.8.

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Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	High	Chemical concentrations were verified with analytical chemistry methods. In order to estimate the actual concentration in the water phase, the following procedures were carried out: The contents of the test flask were filtered through 0.45-mm polycarbonate filters after 72 h of incubation. The filtered water was acidified to pH 0.9 with HCl (500 ml), and 0.4 g NaCl was added in order to enhance the extraction efficiency of the monoesters and phthalic acid. The samples were then extracted with 2.0 ml ethyl acetate, and the organic phase was evaporated to dryness. The residues of phthalic acid and monoesters were dissolved with 200 ml derivatizing reagent and the diesters with 200 ml ethyl acetate containing 20% derivatizing reagent. The possible residues left on the glass surfaces of the test flasks (20-ml glass vials) and associated with algae (and the polycarbonate filters) were dissolved and extracted with ethyl acetate. The extracts were evaporated and derivatized as described previously. When the obtained EC50 values for daphnia or bacteria tests exceeded those obtained for the algal tests, these concentrations were tested with the medium used for daphnia and bacteria tests.
	Metric 10:	Exposure Duration and Frequency	High	48-hr was the appropriate test duration for Daphnia. The 48-h immobilization tests with crustaceans D. magna were performed in accordance with the International Organization for Standardization (ISO) standard
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	A range finding was conducted and final bioassays were done with 5-8 concentrations and control. The test strategy included range-finding tests covering several decades of concentrations and definitive tests to establish EC values and the corresponding 95% confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	Solubility limits were not exceeded for these boiassays.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	No source listed for daphnia
	Metric 14:	Acclimatization and Pretreatment Conditions	High	pH was stated for these bioassays and ISO methods for this 48-hr test were referred to for this study. The ISO methods have ranges for water quality and water formulation. HERO ID 667232
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study reported using and adhering to ISO 6341, Determination of the inhibition of the mobility of Daphnia magna - Acute Toxicity Test. Four replicates were used per concentration and five animals (less than 24 h old at the beginning of the test) were used in each replicate (20 ml test medium per replicate). Each test consisted of five to eight concentrations of test solution and a control group of eight replicates. 11 to 16 concentrations were used with six controls were included in all tests.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Water conditions past pH were not reported, although the study was conducted in accordance with guidelines.

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Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methods were identified and appropriate.
	Metric 18:	Consistency of Outcome Assessment	High	The study assessment was applied consistently. Details of the assessment protocol were reported consistently across treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing was reported in methods or results to indicate differences among treatment or control groups. Although the study authors indicated there were five to eight test concentrations and a control group of eight replicates, the actual concentrations were not reported in the study report.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to suggest differences among groups
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Estimation of LC10 and LC50 methods were reported. Statistical treatment of data was done by probit analysis with maximum likelihood estimation using a standard software program, and results were expressed as EC10 and EC50 values reflecting the immobilization concentrations.
	Metric 22:	Reporting of Data	High	Good representation of results for these bioassays.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: The following 48-hr acute tox test on daphnia was conducted on four target compounds of concern: PA, DBP, BBP, DEHP. The same methods were used for all compounds tested on daphnia. Validation of treatment concentrations was conducted when authors were testing on algae. Although the study authors indicated there were five to eight test concentrations were used, and a control group of eight replicates, the actual concentrations were not reported in the study report.

Overall Quality Determination — High

Study Citation:	Services,, B.I. (2004). Internal report: Alga, growth inhibition test of phthalic acid.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Desmodesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	5353164

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The phthalic acid was identified by CASRN.
	Metric 2:	Test Substance Source	Low	Bayer Chemicals AG is the sponsor of the test, but it was not reported if the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	3 replicates per test concentration and 6 replicates per control
	Metric 5:	Negative Control Response	High	The negative control response was reported in the ”Results” section in an unlabeled table as well as in the ”Summary of Results” section in other unlabeled tables.
	Metric 6:	Randomized Allocation	Low	It was not reported how the algae was allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The one test concentration used in this study was prepared by adding 125.2mg of phthalic acid to 1L of dilution water. This was placed in an ultrasonic bath for one hour on a magnetic stirrer. The test system consisted of 300mL flasks with cotton balls. It was unclear if the test flasks were stirred in any way, and the test volume was uncertain.
	Metric 8:	Consistency of Exposure Administration	Medium	300mL Erlenmeyer flasks were used in the test, but the testing volume was not reported. It was also unclear if cultures were stirred at all during the test. Temperature was maintained in a range from 21-25C with an illumination in the spectral range of 400-700nm. Light intensity was reported to be 4000-8000lx. Initial cell densities were reported to be 10^4 cells/mL.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentration was measured using HPLC and UV/VIS detection, and it was reported that recover rates ranged from 98.1-99.7% of nominal values at 0 hours and from 98.3-100.6% of nominal values at 72h respectively. Therefore, nominal values were used to report results in this paper.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 72h, which is appropriate for an algal growth inhibition test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	There was only one exposure level as study authors reported this was a limit test.
	Metric 12:	Testing at or Below Solubility Limit	High	The exposure concentration was reported to be below the water solubility limit.
Domain 4: Test Organism				

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Study Citation:	Services,, B.I. (2004). Internal report: Alga, growth inhibition test of phthalic acid.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Desmodesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	5353164

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The D. subspicatus was reported to be from The Collection of Algal Cultures of the Institute of Plant Physiology at the University of Gottingen in Germany. It was reported exponentially growing stock cultures were maintained in the test facility to obtain the algal inocula for the test.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Pre-cultures were set up three days prior to the start of the test, and they were reported to be grown under the same conditions as the stock cultures. Inocula were taken from the pre-cultures to initiate the test, which was also conducted under similar condition. There was a slightly different test medium used in the pre-cultures and the test flasks compared to the stock cultures.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Cell densities were reported to be 10^4 cells/mL at test initiation. There were three replicates for the test concentration and six replicates for the negative control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were conducive to organism health. Algal cultures were maintained under constant temperature conditions of 23C with a nutrient medium according to Bringmann and Kuhn. Light intensity was reported to be 4000-8000lx and illumination was continuous.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest– inhibition of growth and growth rate of the algal population.
	Metric 18:	Consistency of Outcome Assessment	High	Cell densities were measured in a microcell counter or by the use of a microscopic counting chamber every 24h for the duration of the study.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	It was reported that a different algal culture medium was used in the stock cultures compared to the pre-cultures and the test flasks. This does not appear to have had a substantial effect on results.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described adequately.
	Metric 22:	Reporting of Data	High	Control and exposure results were reported in the ”Results” section. Tables were not numbered, but results were reported for the control, the 100mg/L concentration, and the 100mg/L without pH adjustment exposure.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected results.

Additional Comments: This evaluation was for the effect of phthalic acid on growth inhibition of the algae Desmodesmus subspicatus. Cell density, growth as an integral of biomass, and growth rate were monitored.

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Study Citation:	Services,, B.I. (2004). Internal report: Alga, growth inhibition test of phthalic acid.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Desmodesmus subspicatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	5353164

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

Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Pseudokirchneriella subcapitata*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	CASRN 88-99-3 and purity >99% were identified
	Metric 2:	Test Substance Source	High	The source as identified and verified at the manufacturer or in the study. The diesters were Merck products, purchased from MerckEurolab (Stockholm, Sweden), and the monoesters were synthesized from phthalic acid anhydride and deficient of the alcohol of interest [4]. In short, the main recipe was as follows:Equimolar amounts of phthalic acid anhydride and the alcoholwere refluxed in toluene. After cooling, the mixture was extractedwith acidified water. The organic phase was concentratedin a rotary evaporator, and the products were recrystallized.Ethyl acetate used for extractions during the chemicalanalyses was of chromatography grade. All other chemicalsused were analytical grade, and MilliQt water (Millipore, Bedford,MA, USA) was used throughout the study.
	Metric 3:	Test Substance Purity	High	Purity was reported (table 1) as >99%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The appropriate control groups were utilized for this bioassay. Each test consisted of 11 to 16 concentrations were made by dilution with ISO freshwater algal test medium. Six controls were included in all tests.
	Metric 5:	Negative Control Response	High	Control growth rates were monitored and reported as 1.6 to 1.8/d for the 72 hr incubations
	Metric 6:	Randomized Allocation	Low	Random allocation was not specifically stated
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Test solution preparation was reported. Miniscale algal growth inhibition tests, with 4 ml test medium in 20-ml glass vials, were conducted according to the ISO standard, briefly described as follows: 11 to 16 concentrations were made by dilution with ISO freshwater algal test medium. Six controls were included in all tests. Potassium dichromate was used as a reference compound. The test flasks were incubated on a shaker (100 rpm) in continuous light (90– 100 mE/m2/s) at 21 6 28C. Samples were withdrawn from each test flask and the controls every 24 h (0, 24, 48, and 72 h).
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent; no inconsistencies were reported. Study was reported as stated in the International Organization for Standardization. 1989. Water quality— Fresh water algal growth inhibition test with Scenedesmus subspicatus and Selenastrum capricornutum. ISO 8692. Geneva, Switzerland

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Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Pseudokirchneriella subcapitata*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	High	Chemical concentrations were verified with analytical chemistry methods. The actual exposure concentration of the phthalates to the algae was determined by chemical analyses carried out in studies performed in parallel to the toxicity tests.
	Metric 10:	Exposure Duration and Frequency	High	72 hr growth bioassay is appropriate for these algae
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The test strategy included range-finding tests covering several decades of concentrations and definitive tests to establish EC values and the corresponding 95% confidence intervals. The standardized toxicity tests with algae were all carried out as concentration–response experiments.
	Metric 12:	Testing at or Below Solubility Limit	High	Solubility limits were not exceeded for these bioassays.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	No source listed for algae
	Metric 14:	Acclimatization and Pretreatment Conditions	High	pH was stated for these bioassays (8.0 -/+ 0.3) and ISO methods for this 72-hr test were referred to for this study. The ISO methods (IS) 8692)have ranges for water quality and water formulation. HERO ID 667212
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study reported using and adhering to ISO 8692. ”Freshwater algal growth inhibition test with unicellular green algae” HERO ID 667212
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Water conditions past pH were not reported although the study was conducted in accordance with guidelines.
	Metric 17:	Outcome Assessment Methodology	High	Outcome assessment methods were identified and appropriate.
	Metric 18:	Consistency of Outcome Assessment	High	The study assessment was applied consistently. Details of the assessment protocol were reported consistently across treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Nothing reported in methods or results to indicate differences among treatment or control groups. Although the study authors indicated there were 11 to 16 test concentrations and 6 controls, the actual concentrations were not reported in the study report.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Nothing reported to suggest differences among groups
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Estimation of LC10 and LC50 methods were reported. Concentration–response curves were described by the Weibull model, which was fitted to data using nonlinear regression applying a computer program developed by Andersen et al. This program also allows for inverse estimations of EC values and the corresponding 95% confidence intervals.

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Study Citation:	Jonsson, S., Baun, A. (2003). Toxicity of mono- and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. Environmental Toxicology and Chemistry 22(12):3037-3043.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Non-vascular Plants; *Pseudokirchneriella subcapitata*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	789536

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	High	Good representation of results for these bioassays.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: The following 72-hr algae inhibition test on Pseudokirchneriella subcapitata was conducted on four target compounds of concern: PA, DBP, BBP, DEHP. The same methods were used for all compounds tested on this algae. Validation of treatment concentrations was conducted. Although the study authors indicated there were 11 to 16 test concentrations and 6 controls, the actual concentrations were not reported in the study report.

Overall Quality Determination High

Study Citation:	Hua, C. P., Xie, Z. K., Wu, Z. J., Zhang, Y. B., Guo, Z. H., Qiu, Y., Wang, L., Wang, Y. J. (2019). The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (Lilium davidii var. unicolor). HortScience 54(2):253-261.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lilium davidii var. unicolor*; Embryo
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6824698

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Test substance identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The test substance was obtained from Tianjin Kexin ChemicalCompany but was not analytically verified.
	Metric 3:	Test Substance Purity	Low	Percent purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The use of a negative control group was reported.
	Metric 5:	Negative Control Response	High	Negative control responses were adequate.
	Metric 6:	Randomized Allocation	Low	Authors did not report how lily bulbs were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Test media preparation details were not given and exposure concentrations were not measured during the study.
	Metric 8:	Consistency of Exposure Administration	Low	Test solution was used to water the soil before planting the bulbs. No other detail was given.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration of 75 days and bulbs were sampled right before the blooming stage. Exposure duration was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Lily bulbs were purchased from lily dealers from lily dealers (Xiguoyuan of Lanzhou City, Gansu Province, China). Bulb size was 3-cm.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Bulbs were refrigerated for 60 days to break dormancy and sterilized by immersing in Imazalil for 1 hr. But unclear whether pretreatment conditions were the the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Medium	5 bulbs in each culture basin and 3 replicates per concentration.

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Study Citation:	Hua, C. P., Xie, Z. K., Wu, Z. J., Zhang, Y. B., Guo, Z. H., Qiu, Y., Wang, L., Wang, Y. J. (2019). The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (Lilium davidii var. unicolor). HortScience 54(2):253-261.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lilium davidii var. unicolor*; Embryo
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6824698

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Soil characteristics were not reported. Bulbs were watered every 2 days and nutrient solution was applied for half a month but unclear whether differences occurred between control and exposed populations
	Metric 17:	Outcome Assessment Methodology	Low	Outcome assessment methodology with respect to bulb sampling was not clearly reported. Also, the outcome assessment methodology of mechanistic endpoints were not given in detail.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted 75 days after planting.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no outcomes unrelated to exposures reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	High	Data for mechanistic endpoints t were presented for all treatment groups and control.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The study did not provide any details on test media preparation and exposure concentrations were not measured. Results were provided only via figures.

Overall Quality Determination — Medium

Study Citation:	Hua, C. P., Xie, Z. K., Wu, Z. J., Zhang, Y. B., Guo, Z. H., Qiu, Y., Wang, L., Wang, Y. J. (2019). The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (Lilium davidii var. unicolor). HortScience 54(2):253-261.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lilium davidii var. unicolor*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6824698

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	Test substance identified as phthalic acid.
	Metric 2:	Test Substance Source	Low	The test substance was obtained from Tianjin Kexin ChemicalCompany but was not analytically verified.
	Metric 3:	Test Substance Purity	Low	Percent purity of the test substance was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The use of a negative control group was reported.
	Metric 5:	Negative Control Response	High	Negative control responses were adequate.
	Metric 6:	Randomized Allocation	Low	Authors did not report how lily bulbs were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Test media preparation details were not given and exposure concentrations were not measured during the study.
	Metric 8:	Consistency of Exposure Administration	Low	Test solution was used to water the soil before planting the bulbs. No other detail was given.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration of 75 days and bulbs were sampled right before the blooming stage. Exposure duration was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Lily bulbs were purchased from lily dealers from lily dealers (Xiguoyuan of Lanzhou City, Gansu Province, China). Bulb size was 3-cm.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Bulbs were refrigerated for 60 days to break dormancy and sterilized by immersing in Imazalil for 1 hr. But unclear whether pretreatment conditions were the the same for control and exposed groups
	Metric 15:	Number of Organisms and Replicates per Group	Medium	5 bulbs in each culture basin and 3 replicates per concentration.
Domain 5: Outcome Assessment				

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Study Citation:	Hua, C. P., Xie, Z. K., Wu, Z. J., Zhang, Y. B., Guo, Z. H., Qiu, Y., Wang, L., Wang, Y. J. (2019). The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (Lilium davidii var. unicolor). HortScience 54(2):253-261.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lilium davidii var. unicolor*; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6824698

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Soil characteristics were not reported. Bulbs were watered every 2 days and nutrient solution was applied for half a month but unclear whether differences occurred between control and exposed populations
	Metric 17:	Outcome Assessment Methodology	Low	Outcome assessment methodology was not clearly reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment conducted 75 days after planting.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Environmental conditions were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no outcomes unrelated to exposures reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed but not described adequately.
	Metric 22:	Reporting of Data	Medium	Data for root length, plant height and fresh weight were presented for all treatment groups and control via figures.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: The study did not provide any details on test media preparation and exposure concentrations were not measured. Results were provided only via figures.

Overall Quality Determination — Medium

Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	"Phthalic acid (purchased from Yifang S&T Ltd. Tianjin, China), dissolved in ethanol, was added to the nutrient solution to concentrations of 0 or 1 mM."
	Metric 2:	Test Substance Source	Low	According to the study authors, phthalic acid was purchased from Yifang S&T Ltd. Tianjin, China.
	Metric 3:	Test Substance Purity	Low	Purity information was not indicated.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls consisted of plants that were exposed to the same solvent concentration and nutrient solution, minus the test substance phthalic acid.
	Metric 5:	Negative Control Response	High	Negative controls showed no signs of distress.
	Metric 6:	Randomized Allocation	Medium	"Each treatment was replicated three times in a completelyrandomized design."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	"When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37× 22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively. The containers were placed in a controlled growth room with a L/D regime of 12/12 h, 25/20°C, and a photon flux density of 140–160 μmol m−2 s−1."
	Metric 8:	Consistency of Exposure Administration	High	Phthalic acid was added to the exposure solution at concentrations of 0 or 1 mM for 5, 10, or 15 days. For length and weight, "At the end of the experiment (15 days after treatment), root and shoot length and fresh and dry weight of seedlings were measured."
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no indication that phthalic acid analytical verification occurred.
	Metric 10:	Exposure Duration and Frequency	High	The duration was long enough and the exposure concentration high enough to measure an effect.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system." There were two treatment groups, 0 or 1 mM, and exposures were for 5, 10, or 15 days. Length and weight were assessed after 15 days.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent ethanol was used in treatment groups.
Domain 4: Test Organism				

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Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	”M. prunifolia, were obtained from Fuping County (34°75’ N, 109°15’ E), Shaanxi Province. Seed sterilization was done according to Zhang et al. (2007) and involved surface-sterilization in 0.3% (v/v) H2O2 for 20 min, followed by several rinses with sterile H2O. The sterilized seeds were stratified at 4°C for 85 days. Sprouted seeds were sown in plastic pots (9 cm in diameter, 12 cmhigh; three seeds per pot) filled with sterilized sand. All pots were placed in a greenhouse at the College of Horticulture, Northwest A&F University, Yangling (34°20’N, 108°24’ E). Plants were grown without supplementary illumination with night and day temperatures at 20 to 25°C and relative humidity at 65–80%. Seedlings were watered once a week with Hoagland nutrient solution (Hoagland 1920), pH 6.0±0.2. When the seedlings reached the sixleaf- stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37×22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively.”
	Metric 14:	Acclimatization and Pretreatment Conditions	High	”Seedlings were allowed to acclimate to the hydroponic conditions for 5 days.”
	Metric 15:	Number of Organisms and Replicates per Group	Medium	45 seeds were transferred for each treatment and control, and the experiment was conducted in triplicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There were no reported issue with housing or acclimation conditions.
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes (length and weight) were observed. Mechanistic endpoints were also assessed.
	Metric 18:	Consistency of Outcome Assessment	Medium	No inconsistencies were indicated for assessing length and weight of plants/seeds.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	”All data were subjected to analysis of variance, followed by Tukey’s Studentized Range Test (SAS Statistical package, version 8.2). Results are presented as the means±standard deviation (SD).”

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Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	High	”The toxic effect of 1 mM phthalic acid appeared after 15 days following treatment. The length of shoot and root of M. prunifolia plants had reduced shoot and root length compared to lower than controls (Fig. 1a). Fresh and dry weights were 3.09 and 1.61 g plant−1 in control plants compared to 2.63 and 0.79 g plant−1 forplants treated with 1 mM phthalic acid (Fig. 1b).”
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported.

Additional Comments: This form is for LENGTH

Overall Quality Determination High

Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	”Phthalic acid (purchased from Yifang S&T Ltd. Tianjin, China), dissolved in ethanol, was added to the nutrient solution to concentrations of 0 or 1 mM.”
	Metric 2:	Test Substance Source	Low	According to the study authors, phthalic acid was purchased from Yifang S&T Ltd. Tianjin, China.
	Metric 3:	Test Substance Purity	Low	Purity information was not indicated.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls consisted of plants that were exposed to same solvent concentration and nutrient solution, minus the test substance phthalic acid.
	Metric 5:	Negative Control Response	High	Negative controls showed no signs of distress.
	Metric 6:	Randomized Allocation	Medium	”Each treatment was replicated three times in a completelyrandomized design”
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	”When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37× 22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively. The containers were placed in a controlled growth room with a L/D regime of 12/12 h, 25/20°C, and a photon flux density of 140–160 μmol m−2 s−1.”
	Metric 8:	Consistency of Exposure Administration	High	Phthalic acid was added to the exposure solution at concentrations of 0 or 1 mM for 5, 10, or 15 days. For length and weight, ”At the end of the experiment (15 days after treatment), root and shoot length and fresh and dry weight of seedlings were measured.”
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no indication that phthalic acid analytical verification occurred.
	Metric 10:	Exposure Duration and Frequency	High	The duration was long enough and the exposure concentration high enough to measure an effect.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	”When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system.” There were two treatment groups, 0 or 1 mM, and exposures were for 5, 10, or 15 days. Length and weight were assessed after 15 days.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent ethanol was used in treatment groups.
Domain 4: Test Organism				

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Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	”M. prunifolia, were obtained from Fuping County (34°75’ N, 109°15’ E), Shaanxi Province. Seed sterilization was done according to Zhang et al. (2007) and involved surface-sterilization in 0.3% (v/v) H2O2 for 20 min, followed by several rinses with sterile H2O. The sterilized seeds were stratified at 4°C for 85 days. Sprouted seeds were sown in plastic pots (9 cm in diameter, 12 cmhigh; three seeds per pot) filled with sterilized sand. All pots were placed in a greenhouse at the College of Horticulture, Northwest A&F University, Yangling (34°20’N, 108°24’ E). Plants were grown without supplementary illumination with night and day temperatures at 20 to 25°C and relative humidity at 65–80%. Seedlings were watered once a week with Hoagland nutrient solution (Hoagland 1920), pH 6.0±0.2. When the seedlings reached the sixleaf- stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37×22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively.”
	Metric 14:	Acclimatization and Pretreatment Conditions	High	”Seedlings were allowed to acclimate to the hydroponic conditions for 5 days.”
	Metric 15:	Number of Organisms and Replicates per Group	Medium	45 seeds were transferred for each treatment and control, and the experiment was conducted in triplicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There were no reported issue with housing or acclimation conditions.
	Metric 17:	Outcome Assessment Methodology	High	The intended outcomes (length and weight) were observed. Mechanistic endpoints were also assessed.
	Metric 18:	Consistency of Outcome Assessment	Medium	No inconsistencies were indicated for assessing length and weight of plants/seeds.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	”All data were subjected to analysis of variance, followed by Tukey’s Studentized Range Test (SAS Statistical package, version 8.2). Results are presented as the means±standard deviation (SD).”

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Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	High	”The toxic effect of 1 mM phthalic acid appeared after 15 days following treatment. The length of shoot and root of M. prunifolia plants had reduced shoot and root length compared to lower than controls (Fig. 1a). Fresh and dry weights were 3.09 and 1.61 g plant−1 in control plants compared to 2.63 and 0.79 g plant−1 forplants treated with 1 mM phthalic acid (Fig. 1b).”
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported.

Additional Comments: This form is for WEIGHT

Overall Quality Determination High

Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	”Phthalic acid (purchased from Yifang S&T Ltd. Tianjin, China), dissolved in ethanol, was added to the nutrient solution to concentrations of 0 or 1 mM.”
	Metric 2:	Test Substance Source	Low	According to the study authors, phthalic acid was purchased from Yifang S&T Ltd. Tianjin, China
	Metric 3:	Test Substance Purity	Low	Purity information was not indicated.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls consisted of plants that were exposed to same solvent concentration and nutrient solution, minus the test substance phthalic acid.
	Metric 5:	Negative Control Response	High	Negative controls showed no signs of distress
	Metric 6:	Randomized Allocation	Medium	”Each treatment was replicated three times in a completelyrandomized design”
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	”When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37× 22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively. The containers were placed in a controlled growth room with a L/D regime of 12/12 h, 25/20°C, and a photon flux density of 140–160 μmol m−2 s−1.”
	Metric 8:	Consistency of Exposure Administration	High	Phthalic acid was added to the exposure solution at concentrations of 0 or 1 mM for 5, 10, or 15 days.
	Metric 9:	Measurement of Test Substance Concentration	Low	There was no indication that phthalic acid analytical verification occurred.
	Metric 10:	Exposure Duration and Frequency	High	The duration was long enough and the exposure concentration high enough to measure an effect.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	”When the seedlings reached the sixleaf-stage, batches of 45 uniform seedlings were transferred into a hydroponic system.” There were two treatment groups, 0 or 1 mM, and exposures were for 5, 10, or 15 days.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent ethanol was used in treatment groups.
Domain 4: Test Organism				

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Study Citation:	Bai, R., Ma, F. W., Liang, D., Zhao, X. (2009). Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of Malus prunifolia. Journal of Chemical Ecology 35(4):488-494.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	”M. prunifolia, were obtained from Fuping County (34°75’ N, 109°15’ E), Shaanxi Province. Seed sterilization was done according to Zhang et al. (2007) and involved surface-sterilization in 0.3% (v/v) H2O2 for 20 min, followed by several rinses with sterile H2O. The sterilized seeds were stratified at 4°C for 85 days. Sprouted seeds were sown in plastic pots (9 cm in diameter, 12 cmhigh; three seeds per pot) filled with sterilized sand. All pots were placed in a greenhouse at the College of Horticulture, Northwest A&F University, Yangling (34°20’N, 108°24’ E). Plants were grown without supplementary illumination with night and day temperatures at 20 to 25°C and relative humidity at 65–80%. Seedlings were watered once a week with Hoagland nutrient solution (Hoagland 1920), pH 6.0±0.2. When the seedlings reached the sixleaf- stage, batches of 45 uniform seedlings were transferred into a hydroponic system (plastic container; 45×37×22.5 cm) filled with 5 l half Hoagland nutrient solution at pH 6.0±0.2 and electrical conductivity at 1.2 ms/cm, respectively.”
	Metric 14:	Acclimatization and Pretreatment Conditions	High	”Seedlings were allowed to acclimate to the hydroponic conditions for 5 days.”
	Metric 15:	Number of Organisms and Replicates per Group	Medium	45 seeds were transferred for each treatment and control, and the experiment was conducted in triplicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There were no reported issue with housing or acclimation conditions.
	Metric 17:	Outcome Assessment Methodology	High	Mechanistic outcomes (ie oxidative stress) were observed via measurements of multiple mechanistic pathways. Mechanistic endpoints were also assessed. These included free radical MDA, H2O2, and O2 measurement as well as antioxidant enzymes (SOD, POD, and CAT) activity measurement.
	Metric 18:	Consistency of Outcome Assessment	Medium	”Root samples were taken from both control and phthalic acid-treated plants on days 5, 10, and 15 after treatment, and the tissue was frozen in liquid nitrogen and stored at −70°C until analysis.”
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups in environmental conditions or other factors that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				

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Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6813707

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	”All data were subjected to analysis of variance, followed by Tukey’s Studentized Range Test (SAS Statistical package, version 8.2). Results are presented as the means±standard deviation (SD).”
	Metric 22:	Reporting of Data	High	’Lipid peroxidation was followed by measuring MDA accumulation using the method of Baziramakenga et al. (1995) with some modifications.....Samples were centrifuged at 12,000×g for 5 min, and then the absorbance of thesupernatant was measured at 450, 532, and 600 nm.””H2O2 in the supernatant was measured according to Patterson et al. (1984).....Absorbance at 410 nm was measured, and the H2O2 concentration was calculated according to a standard curve.””The rate of O2 · generation was measured as described by Elstner and Heupel (1976) with some modifications.....The final solution was mixed with an equal volume of chloroform and the absorbance of the pink phase was measured at 530 nm.””Antioxidant enzymes (SOD, POD, and CAT) were extracted according to the method of Yu et al. (2003) with some modifications.””Superoxide dismutase activity was measured according to Beauchamp and Fridovich (1971) with minor modification.....The reaction was initiated andterminated by turning the light on and off, respectively. The A560 was measured on a spectrophotometer and tubes containing the assay mixture, but without the root extract (control), were illuminated to determine maximum A560.””Peroxidase activity was measured according to Sofo et al. (2004) with some modification....The Increase in A470nm due to the oxidation of guaiacol was measured at 20°C.””Catalase activity was assayed by monitoring the decrease in A240nm (Aebi 1984).””AsA-related enzymes were extracted according to Nakano and Asada (1981)....The homogenate was centrifuged (16,000×g for 15 min at 2°C) and the supernatant was used for APX, GR, MDHAR, and DHAR analyses.””Ascorbate peroxidase was measured by monitoring the decrease in A290 nm (Nakano and Asada 1981).””Glutathione reductase activity was monitored atA340 nm in a 1 ml reaction mixture containing Tris–HCl (100 mM; pH 8.0), ethylenediaminetetraacetic acid (1 mM), oxidized glutathione (GSSG;1 mM), and NADPH (0.2 mM). The reaction was initiated by adding NADPH (Grace and Logan 1996).””Monodehydroascorbate reductase activity was assayed at 340 nm in a 1 ml reaction mixture containing Hepes–KOH (50 mM; pH 7.6), NADH (0.1 mM), AsA (0.25 mM), and AsA oxidase (EC 1.10.3.3; 0.25 U). The reaction was initiated by adding AsA oxidase (Miyake and Asada 1992).””Dehydroascorbate rductase activity was measured at 265 nm in a 1 ml assay solution containing Hepes–KOH (100 mM; pH 7.0), ethylenediaminetetraacetic acid (1 mM), GSH (2.5 mM), and DHA (0.2 mM). The reaction was initiated by adding DHA (Dalton et al. 1986).”
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported.

Additional Comments: This form is for MECHANISTIC endpoints

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Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Malus prunifolia (crabapple)*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	Phthalic acid
HERO ID:	6813707

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

Study Citation:	Huiyong, Y., Hongbo, L., Guoming, S., Sampietro, D. A., Xinxin, G. (2014). Effects of allelochemicals from tobacco root exudates on seed germination and seedling growth of tobacco. Allelopathy Journal 33(1):107-119.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Vascular Plants; *Nicotiana tabacum*; ZhongYan 104; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6968271

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	The paper reports the exposure of ”phthalic acid” within the results and discussion on page 8/13. No CAS number or nomenclature provided.
	Metric 2:	Test Substance Source	Low	It is unclear the source of the ”Phthalic acid” used in this study. If extracted from the previous study in the paper OR obtained from a manufacturer.
	Metric 3:	Test Substance Purity	Low	Nothing on the purity was detailed.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls (just water) were noted in the study.
	Metric 5:	Negative Control Response	Medium	Results from treatment concentrations are presented as a % of control response. This presentation of data makes it difficult to observe the magnitude of the control response.
	Metric 6:	Randomized Allocation	Low	No random allocation noted.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Test media preparation details were very limited. Concentration of test substance was not measured during the study.
	Metric 8:	Consistency of Exposure Administration	High	Administration of treatment concentrations and control appear to be consistent.
	Metric 9:	Measurement of Test Substance Concentration	Low	Nominal concentrations were reported but not verified.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was for 7 days but there were no details on exposure frequency other than the initial application of the test substance into the petri dish.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Nominal concentrations are reported as 0.1, 0.25, and 0.5 g/L.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations <0.5 g/L are below solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source for the ZhongYan 104 variety used in the germination experiments were not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions of the seeds were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	50 seeds per concentration with 20 assessed for growth metrics. three replicates (page 4/13).

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Study Citation:	Huiyong, Y., Hongbo, L., Guoming, S., Sampietro, D. A., Xinxin, G. (2014). Effects of allelochemicals from tobacco root exudates on seed germination and seedling growth of tobacco. Allelopathy Journal 33(1):107-119.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Vascular Plants; *Nicotiana tabacum*; ZhongYan 104; Embryo
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6968271

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Light and temp conditions were reported, but no other details on water conditions for each treatment were detailed.
	Metric 17:	Outcome Assessment Methodology	Low	Methods for dry weight and root length were not described.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appear to be reported consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information presented on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis (ANOVA w/Tukey's post-hoc) was reported.
	Metric 22:	Reporting of Data	Medium	Data presented as a % of control.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Data presented as % of control with no variance reported.

Additional Comments: The source and purity of the chemical was not reported or clear. Preparation of treatment solutions was not detailed. Data was presented as % of control response with no variance reported.

Overall Quality Determination — Uninformative

Study Citation:	Huiyong, Y., Hongbo, L., Guoming, S., Sampietro, D. A., Xinxin, G. (2014). Effects of allelochemicals from tobacco root exudates on seed germination and seedling growth of tobacco. Allelopathy Journal 33(1):107-119.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Vascular Plants; *Nicotiana tabacum*; ZhongYan 104; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Phthalic acid
HERO ID:	6968271

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Uninformative	The paper reports the exposure of "phthalic acid" within the results and discussion on page 8/13. No CAS number or nomenclature provided.
	Metric 2:	Test Substance Source	Low	It is unclear the source of the "Phthalic acid" used in this study. If extracted from the previous study in the paper OR obtained from a manufacturer.
	Metric 3:	Test Substance Purity	Low	Nothing on the purity was detailed.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls (just water) were noted in the study.
	Metric 5:	Negative Control Response	Medium	Results from treatment concentrations are presented as a % of control response. This presentation of data makes it difficult to observe the magnitude of the control response.
	Metric 6:	Randomized Allocation	Low	No random allocation noted.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Test media preparation details were very limited. Concentration of test substance was not measured during the study.
	Metric 8:	Consistency of Exposure Administration	High	Administration of treatment concentrations and control appear to be consistent.
	Metric 9:	Measurement of Test Substance Concentration	Low	Nominal concentrations were reported but not verified.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of exposure was for 7 days but there were no details on exposure frequency other than the initial application of the test substance into the petri dish.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Nominal concentrations are reported as 0.1, 0.25, and 0.5 g/L.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations <0.5 g/L are below solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The source for the ZhongYan 104 variety used in the germination experiments were not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether pretreatment conditions of the seeds were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Fifty seeds per concentration with germination percentage calculated by counting the number of germinated seeds out of 50. Three replicates were used (page 4/13).
Domain 5: Outcome Assessment				

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Study Citation:	Huiyong, Y., Hongbo, L., Guoming, S., Sampietro, D. A., Xinxin, G. (2014). Effects of allelochemicals from tobacco root exudates on seed germination and seedling growth of tobacco. Allelopathy Journal 33(1):107-119.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Vegetation; Vascular Plants; *Nicotiana tabacum*; ZhongYan 104; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	Phthalic acid
HERO ID:	6968271

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Light and temp conditions were reported, but no other details on water conditions for each treatment were detailed.
	Metric 17:	Outcome Assessment Methodology	Medium	Germination was reported as being assessed but no details on the specific methods.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes appear to be reported consistently among treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study did not provide enough information to allow a comparison of environmental conditions across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information presented on outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis (ANOVA w/Tukey's post-hoc) was reported.
	Metric 22:	Reporting of Data	Medium	Data presented as a % of control (Figure 2).
	Metric 23:	Explanation of Unexpected Outcomes	Low	Data presented as % of control with no variance reported.

Additional Comments: This evaluation is for the germination percentage. The source and purity of the chemical was not reported or clear. Preparation of treatment solutions was not detailed. Data was presented as % of control response with no variance reported.

Overall Quality Determination — Uninformative

Study Citation:	Loffredo, E., Traversa, A. (2014). Soil and compost humic fractions regulate the response of Sclerotinia sclerotiorum to exogenously added allelochemical compounds. Biology and Fertility of Soils 50(8):1281-1290.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Other; Fungus; *Sclerotinia sclerotiorum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6826077

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as phthalic acid and was definitively identified with proper nomenclature and chemical structure (benzene-1,2-dicarboxylic acid). Form and other identifying characteristics were not provided. Chemical name was misspelled throughout the study report.
	Metric 2:	Test Substance Source	Low	Phthalic acid was obtained from Sigma-Aldrich srl, Milano, Italy but was not analytically verified.
	Metric 3:	Test Substance Purity	High	The percent purity was reported as 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control was used in the experiment. Negative control contained potato dextrose agar as a growth medium.
	Metric 5:	Negative Control Response	High	Control responses were adequate.
	Metric 6:	Randomized Allocation	Low	The authors did not report on how the organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The test media preparation details were not reported and the effects of autoclaving on the test substance was not studied. Exposure concentration was not measured during the study.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure administration details were not clearly reported. The amount of medium poured into each petri dish was not given.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured. The culture medium with phthalic acid was autoclaved prior to the inoculation with the test organisms. No quantification was done to check whether the phthalic acid concentration degraded as a result of the autoclaving, the authors insisted that no effect was observed, but this was based on a personal communication and no further information was given.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was 80 hours.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Only one exposure concentration was used.
	Metric 12:	Testing at or Below Solubility Limit	High	Exposure concentrations were below the water solubility limit.
Domain 4: Test Organism				

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Study Citation:	Loffredo, E., Traversa, A. (2014). Soil and compost humic fractions regulate the response of Sclerotinia sclerotiorum to exogenously added allelochemical compounds. Biology and Fertility of Soils 50(8):1281-1290.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Other; Fungus; *Sclerotinia sclerotiorum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6826077

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The source of S. sclerotiorum strain was reported and fresh subcultures were used for the study. "The S. sclerotiorum strain was isolated from a greenhouse grown eggplant and grown on potato dextrose agar (PDA, Oxoid) in Petri dishes at 20 °C in the dark. Fresh subcultures of the fungus were obtained from 2-mm PDA disks overgrown by 5-day mycelium collected from the growing margin of the primary colony."
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Pre-treatment conditions were not reported.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	5 replicates were used.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Petri dishes were kept in the dark at a constant temperature (20 degrees C).
	Metric 17:	Outcome Assessment Methodology	Medium	Fungal growth assessment methods were not well described. The fungal growth effects were based on, "the apparent morphology and radial growth, in millimetres, of the mycelium wereevaluated." Number of sclerotia were also counted and used as a proxy for growth effects.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The study does not provide enough information to allow for a comparison of confounding variables. This is significant because of the significant growth enhancement observed for several of the chemicals.
	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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	One-way ANOVA was used.
	Metric 22:	Reporting of Data	Low	Growth data was reported as percent variation relative to the control and presented only as figures.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: Fungal growth assessment method was not reported and the growth data were presented as percent variation relative to the control.

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Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; Cell Culture Media; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Other; Fungus; *Sclerotinia sclerotiorum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	Phthalic acid
HERO ID:	6826077

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